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...OL. VI DECEMBER, 1918 No. 6

SOIL SCIENCE

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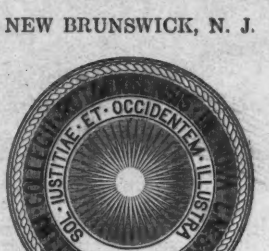

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PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

THE CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, LONDON, E. C.

Entered as second-class matter August 23, 1917, at the post office at Baltimore, Maryland, under the act of March 3, 1879.

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Price { $6.00 per year, two volumes, United States
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$6.50 per year, two volumes, other countries

SOIL SCIENCE

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THE RELATION OF THE LIME REQUIREMENTS OF SOILS TO THEIR RETENTION OF AMMONIA[1]

L. P. HOWARD

Rhode Island Agricultural Experiment Station

Received for publication November 23, 1918

Several methods for determining the lime requirements of soils have been developed recently. New terms are being coined rapidly to designate various types of acidity which the methods propose to measure. A "lime requirement" determined by one method, in general may bear little relation to that secured by another.

The total amount of base removed by a soil from a solution of a neutral or alkaline salt is the resultant of at least two reactions proceeding simultaneously. These may be designated as (a) chemical fixation, and (b) physical absorption.

The number of basic ions removed from solutions of different bases will differ. In the first place, that removed for the neutralization of free acid is the same for various bases; but in many of our soils, the general idea is that the major portion of the base is retained by insoluble acid salts. Here an equilibrium is established. The extent to which the neutralization of these acid constituents proceeds before equilibrium is established depends upon (a) the activity of the base, or the extent to which it is ionized, (b) concentration, (c) temperature, and other factors.

What seems to be desired in a method for measuring "lime requirements" by the absorption of bases is that the neutralization of free acids shall be complete, and that the neutralization of the acid silicates shall proceed to about the same point for soils of quite different character before equilibrium is established.

It was this idea which prompted the work herein recorded. A study of various procedures has been made to see whether one could be developed which would give a "requirement" that would be, within reasonable limits, independent of (a) concentration of the base, (b) temperature, and (c) time of contact.

In adopting a base which would neutralize insoluble acid residues to about the same extent for soils of different reaction, the idea was held that to satisfy

[1] Contribution 227 from the Agricultural Experiment Station of the Rhode Island State College, at Kingston, R. I. Read at the 1916 Convention of the Association of Official Agricultural Chemists.

this condition there must be established, at the end of the reaction, circumstances in which no excess base should remain.

A volatile base, as ammonium hydroxide, suggested itself. The principle of the procedure was to treat the soil with ammonia water, evaporate to dryness, and measure the retained ammonia.

The procedure in detail as finally adopted, together with the effect of certain variations upon the absorption, are here recorded.

DISTILLATION BY AERATION

The aeration method seemed to possess desirable features for estimating small amounts of ammonia. However, as there is considerable discussion as to the virtues of this method, certain features had to be investigated. These were (a) selection of alkali, (b) aeration necessary, and (c) completeness of ammonia absorption.

a. Selection of alkali. Sodium carbonate, sodium hydroxide and magnesium oxide were used with the following results:

ALKALI USED	NH_3 RECOVERED			
	Absorption bottles		Blank soil	Corrected recovery
	No. 1	No. 2		
	mgm.	*mgm.*	*mgm.*	*mgm.*
Na_2CO_3 (5 gm.)	51.34	−0.08	0.46	50.88
NaOH (2 gm.)	53.99	0.05	1.88	52.11
MgO (5 gm.)	48.68	−0.05		48.68
MgO (5 gm.) (distilling by boiling)	51.14		1.21	49.93

The use of NaOH permitted a somewhat more rapid recovery of ammonia, but the amount evolved was subject to greater variation than that recorded by the use of Na_2CO_3.

Distillation by boiling with MgO yielded results quite similar to those obtained by the use of sodium carbonate. The latter however, seemed to work very satisfactorily and was used as the alkali in connection with the results to be recorded.

b. Aeration necessary. With sodium carbonate as much ammonia was obtained by 18 hours aeration as by 60 hours, and the former period was adopted. Six determinations were conducted in a string and a current of 300 liters of air per hour [measured by the Kober method (1)] passed through the last flask.

c. Completeness of ammonia absorption. The figures recorded under (a) "Selection of alkali" indicate that the absorption is complete in the first bottle; that is, for amounts of ammonia up to 75 mgm., with the rate of aeration indicated.

These results indicate very clearly that the reaction which takes place between the soil and an aqueous solution of ammonia is rapid and is complete in one hour.

Small variations in temperature do not appreciably affect the amount of ammonia retained. However, at the lower temperature it is much more difficult to secure duplicate determinations. Evidently at these temperatures

TABLE 1

Time of contact before evaporation, and its influence upon ammonia retained. (170 mgm. of ammonia added to 25 gm. of surface soil. Evaporated at 95–98° C.)

RHODE ISLAND PLAT	½ HOUR	1 HOUR	4 HOURS	10 HOURS
No. 23: $(NH_4)_2$ SO_4, unlimed*				
NH_3 retained (mgm.)		52.56	52.08	52.97
†Lime requirement (pounds CaO)		6,925	6,862	6,979
No. 25: $(NH_4)_2$ SO_4, limed,*				
NH_3 retained (mgm.)		39.84	40.12	41.20
† Lime requirement (pounds CaO)		5,249	5,286	5,428
No. 27: $NaNO_3$, unlimed*				
NH_3 retained (mgm.)		41.95	42.70	43.04
†Lime requirement (pounds CaO)		5,527	5,428	5,670
No 29: $NaNO_3$, limed				
NH_3 retained (mgm.)		28.35	29.44	28.49
*Lime requirement		3,735	3,879	3,753
No. 30: corn acre, rye plat‡				
NH_3 retained (mgm.)	18.56	19.38		
†Lime requirement (pounds CaO)	2,441	2,573		

*Surface soil, 8 inches deep, collected June 29, 1916.

†Requirement of 2,000,000 pounds soil.

‡Soil 12 inches deep, collected June 10, 1915, evaporated at 60° to 65° C.

TABLE 2

Retention of ammonia dependent upon temperature of evaporation. [25 gms. soil, 170 mgm. ammonia (volume 50 cc.), 1 hour contact]

	65–75° C.		95–98° C.	
	NH_3 retained	Lime requirement*	NH_3 retained	Lime requirement*
	mgm.	*lbs.*	*mgm.*	*lbs.*
Plat 23	52.56	6,925	52.22	6,880
Plat 25	40.66	5,357	39.98	5,267
Plat 27	43.38	5,715	43.79	5,769
Plat 29	30.39	4,007	28.08	3,699
Corn acre rye plat	19.92	2,629	17.68	2,329

*Requirement of 2,000,000 pounds of soil in pounds CaO.

more ammonia is held by physical forces. Combinations with very weak organic acids might exist, which would decompose at the higher temperature.

In the smaller application of ammonia quite wide variation in retention results. In amounts of 170 to 340 mgm., the influence upon retention is practically negligible.

TABLE 3

Retention dependent upon mass of ammonia added.. (25 gm. soil, 1 hour contact, temperature 95–98° C.)

	85 MGM. NH_3 ADDED		170 MGM. NH_3 ADDED		255 MGM. NH_3 ADDED		340 MGM. NH_3 ADDED	
	NH_3 retained	Lime requirement*	NH_3 retained	Lime requirement*	NH_3 retained	Lime requirement*	NH_3 retained	Lime requirement*
	mgm.	*lbs.*	*mgm.*	*lbs.*	*mgm.*	*lbs.*	*mgm.*	*lbs.*
Plat 23	45.49	5,993	51.74	6,817	53.04	6,988	53.38	7,033
Plat 25	32.91	4,336	39.16	5,159	40.52	5,338	40.18	5,294
Plat 27	33.45	4,407	43.65	5,751	43.04	5,670	42.77	5,635
Plat 29	22.37	2,947	26.77	3,527	27.47	3,619	28.49	3,753

	70 MGM. NH_3 ADDED		140 MGM. NH_3 ADDED		210 MGM. NH_3 ADDED		280 MGM. NH_3 ADDED	
	NH_3 retained	Lime requirement†	NH_3 retained	Lime requirement†	NH_3 retained	Lime requirement†	NH_3 retained	Lime requirement†
	mgm.	*lbs.*	*mgm.*	*lbs.*	*mgm.*	*lbs.*	*mgm.*	*lbs.*
Corn acre, rye plat†	19.38	2,573	20.74	2,732	23.12	3,046	23.12	3,046

*Requirement of 2,000,000 pounds of soil in pound CaO.

†Temperature 60 to 65° C.

METHOD

Treat 25 gm. of soil in an evaporating dish with 50 cc. N/5 NH_4OH. Stir the mixture occasionally during a period of one hour. Evaporate the solution to dryness on a water bath containing boiling water. Rub the soil up with a pestle and allow it to remain upon the bath for 1½ hours. Wash the soil into a 500 cc. Kjeldahl flask with 100 cc. of ammonia-free water. Add 5 to 10 gm. of sodium carbonate and distil by the aeration method [essentially the method used by Potter and Snyder (2)], keeping the flasks perpendicular and the bottom of the distillation tube slightly above the bottom of the flask. Absorb the liberated ammonia in 25 cc. N/5 H_2SO_4 diluted to 200 cc. Determine the excess acid by titration with N/25 NH_4OH, using alizarin red as indicator. Make blank determinations with each soil.

DISCUSSION OF METHOD

The procedure as outlined yields a requirement which is only slightly affected by wide variation in the conditions under which retention takes place. No other method tried by the writer begins to be so free from analytical weaknesses as the procedure outlined. In other soil types, however, it may fail absolutely to qualify even from a laboratory standpoint.

The real test, however, is what it will do in the way of indicating small variations in reaction, under field conditions.

No definite correlation with crop yields can be offered at this time. It had been hoped that the method may be submitted to a comparison with a crop sensitive to small variations in acidity, but such data cannot be presented now.

A few laboratory experiments have been made to learn to what extent the retention of ammonia can be used as an index of the absorption for other bases.

SUBSTITUTION OF BASES

The plan was to evaporate to dryness the soil treated with solutions of various alkalies and alkaline earths and record the effect upon ammonia retention. This was done in the case of sodium and potassium in the hydroxides, carbonates and chlorides, and with calcium and barium in the hydroxides.

The results indicated that the amount of ammonia retained was diminished in practically equivalent amounts when the soil was acted upon by the hydroxides and carbonates of sodium or potassium. It was diminished very slightly by the chlorides.

With barium and calcium hydroxide, the ammonia absorption was diminished only about one-half what it should have been when figured on the equivalent basis. Their reaction was much slower than that of the alkalies, and it was probably not complete. Some of the solution was probably carbonated and rendered inactive and would thus neither enter into combination with the soil nor by force of its alkalinity drive off its equivalent amount of ammonia.

In general, those bases which tend to cause deflocculation and dissolve organic matter are held in equivalent amounts as measured by the subsequent ammonia retention, while those bases which cause flocculation and become carbonated in the process of evaporation do not react in this way.

ABSORPTION OF AMMONIA BY CARBON BLACK

The well-known power of finely divided particles to hold gases upon their surfaces by physical forces led to the question of what the absorptive power of a substance like carbon black would be under the conditions existing in the method.

Consequently, 45 mgm. of ammonia in a volume of 50 cc. were evaporated from 5 gm. of carbon black. A blank determination of ammonia in the carbon black itself was made after evaporating with 50 cc. water.

	AMMONIA RECOVERED
	mgm.
Carbon Black, blank	6.59
Carbon Black + ammonia	5.84

It is seen that no ammonia is held under these conditions and it is believed therefore that little ammonia is retained physically by the soils under similar treatment.

Comparison with the Veitch method

	REQUIREMENT POUNDS CaO PER 2,000,000 POUNDS SOIL	
	Ammonia method	Veitch method
Plat 23	6,925	8,714
Plat 25	5,249	5,810
Plat 27	5,527	8,070
Plat 29	3,735	4,842

The requirement indicated by the ammonia method runs consistently lower, the greatest variation being in the case of soil from plat 27. The requirement indicated by the Veitch method for this soil is comparatively too high as measured by crop yields.

Soils treated according to the Veitch method yield an aqueous extract more alkaline than the extract of soils after evaporation with ammonia, which is nearly neutral.

ABSORPTION OF AMMONIA BY SOILS

After the completion of this paper there appeared in the literature a publication by Cook treating of the "Absorption of Ammonia by Soils" (3). The purpose of the present paper did not permit a review of the theories relative to ammonia absorption. The paper referred to makes such a contribution.

In the course of that paper it was pointed out that the application of calcium and sodium hydroxide resulted in an increased ammonia absorption in all cases but one. In the present paper it is shown that a decrease in retention of ammonia results from each of these treatments. It is desired merely to emphasize at this time that the method of procedure was entirely different, as given in two papers. Cook was working with solutions of ammonium sulfate at room temperature, while in the present work ammonium hydroxide was used and the excess eliminated at the temperature of boiling water.

The equilibrium that is established under Cook's treatment is referred to as being dependent upon numerous factors, among which are physical, physicochemical, chemical and perhaps biological. The absorption which results from his procedure indicates that these factors are active.

In the present paper it is pointed out that the retention of ammonia which results from the equilibrium established by the procedure outlined is dependent upon fewer factors and seems to follow the direction of true chemical reaction.

Attention is called to Cook's work, and especially to the difference in procedure, lest a hasty review of his paper should give the idea that it is incompatible to use the "Retention of Ammonia" as an index of lime requirements.

SUMMARY AND CONCLUSIONS

A procedure is outlined for determining the lime requirements of soils, which consists in treating the soils with ammonium hydroxide, evaporating off the excess ammonia at a temperature of boiling water, and estimating the retained ammonia.

Within reasonable limits the requirement based upon this retention was independent of (a) concentration of ammonia added, (b) time of contact, and (c) temperature during evaporation.

The requirement was about 25 per cent lower than that indicated by the Veitch method.

Aeration in the presence of sodium carbonate for 18 hours (300 liters of air per hour) is sufficient to remove completely 50 to 75 mgm. of ammonia from the soil.

Sodium, ammonium and potassium from solutions of their hydroxides and carbonates are retained in practically equivalent amounts.

It is believed that the ammonia retained is held chemically by a neutralization of either free acids, acid organic compounds or acid salts, while physical absorption is largely prevented.

The "requirement" based upon the ammonia retention agreed in general with field observations, to the extent that soils needing the most lime showed the greatest "requirement."

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THE EFFECT OF ORGANIC MATTER ON SOIL REACTION

R. E. STEPHENSON

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Received for publication December 1, 1918

INTRODUCTION[1]

Organic material, which is largely protein in nature, is very complex in composition, and its destruction results in many complicated reactions, both synthetic and analytic. The external factors which effect these reactions are subject to continuous fluctuation and hence equilibrium probably never exists. It would seem quite reasonable to assume that in general the reactions follow certain general tendencies. For example there may be a periodic production of acidity or of basicity, or of both, depending upon the stage of decomposition. This work has been planned to study the effect of organic matter on the production of acids or bases in the soil.

Very little work along this line has been reported. White (20,21) of Pennsylvania carried out pot experiments on the effect of poultry and stable manure and fresh and dried green manures upon soil acidity (Veitch Method), using the common farm crops and sorrel as green manures. A considerable reduction in limestone requirement was shown during the first two weeks, but this reduction might have resulted from the mere addition of the organic materials and not been due to decomposition processes at all. Red clover was found to be the best corrective of acidity, while wheat and corn increased the lime requirement more than any of the other materials. Changes in reaction during the second and third months seem to have been very slight. In general the effects produced were quite varied and fluctuating.

Skinner and Beattie (17) studied the effect of certain mineral fertilizers as well as organic materials, upon soil reaction. They found that sulfates increased the acidity, while organic matter had a varying effect. Starch increased the acidity. Manure caused only a slight increase, and leachings from manure caused less acidity than was present in untreated soil. They conclude that the nature of the decomposition of the organic material in the soil and the character of the life processes in the soil affects the influence of such substances on soil reaction.

Miller of Missouri (13) reports that manure, wheat, straw, green oats, dry oats, and clover in the soil caused a decrease in soil acidity for the first 8

[1] Acknowledgments: This work was planned in consultation with Dr. P. E. Brown and Dr. R. S. Potter to whom credit is due for many helpful suggestions and criticisms.

weeks, after which there was a gradual increase until at the end of 26 weeks, the treated soils were as acid as the untreated. The experiment indicates that ordinary green crops and manure turned under do not increase acidity, but that the use of a sugar-containing crop, such as sorghum, may have this effect for a few weeks.

These reports give the only available results bearing directly upon the subject, and in general they indicate that the effect upon soil reaction depends very much upon the nature of the organic matter used.

THE COMPOSITION OF ORGANIC MATTER

Nitrogenous material

There are two kinds of organic material to be considered here, nitrogenous and carbohydrates. The nitrogenous materials consist principally of proteins or their degradation products. A small amount of nitrogen may of course be in the form of nitrate or ammonium salts, some in the form of nitrogen bases, including alkaloids, which are the basic substances of plants; ptomaines, basic substances of animal origin, and other products representing the various stages of protein decomposition. Purine and pyrimidine bases some of which have been isolated from soils, may arise from the breaking down of nucleic acids; and choline, also a strong base, is a product of lecithin disintegration. These are the principal sources of nitrogen other than proteins.

The breaking down of protein materials is a hydrolytic process. The first nitrogen liberated is probably that from the amide groups. Then further decomposition leads to breaking of the amide linkages, and the production of free amino acids. These acids are an easily available source of energy for many bacteria and molds and are further decomposed into bases and acids. The particular kind of decomposition depends upon aeration and other factors. There may be either a decarboxylation or deaminization or both processes may occur. In general anaerobic bacteria reduce alpha-amino acids forming saturated fatty acids and ammonia. Aerobic bacteria tend to oxidize alpha-amino acids to a fatty acid containing one less carbon atom, producing at the same time carbon dioxide and free ammonia. Four type reactions which illustrate the different decomposition processes most likely to occur are given below as taken from Dakin (4).

1. $RCH_2 - CHNH_2 - COOH + H_2$	$R - CH_2CH_3COOH + NH_3$ (Reduction)
2. $RCH_2 - CHNH_2 - COOH + O_2$	$R - CH_2COOH + NH_3 + CO_2$ (Oxidation)
3. $RCH_2 - CHNH_2 - COOH + H_2O$	$R - CH_2OH + NH_3 + CO_2$ (Hydrolysis)
4. $RCH_2 - CHNH_2 - COOH$	$RCH_2\ CH_2NH_2 + CO_2$ (Decarboxylation)

Other kinds of reaction might occur, and these acids, amines and alcohols are of course, further decomposed. Such reactions though not studied originally from the soils standpoint show very well the general nature of processes likely to be occurring in the soil.

As the decomposition of protein proceeds, therefore, it is entirely possible for acidity to increase quite markedly. The glycine and alanine that is split off may produce acetic and propionic acids, serine may yield propionic and formic acids; valine and leucine, isovalerianic, and isovaleric acids; and other amino acids may likewise yield an organic acid. In this study a strong odor of butyric acid was evident in the first stages of decomposition. At the same time basic compounds may be produced and these nitrogen bases may likewise be ammonified. Ammonia will, of course neutralize acids, as will also other nitrogen bases. These, in the absence of mineral bases would tend to permit nitrification, but the production of nitric acid would again make further demands for base. At the same time, so far as results show, the organic acids which might have accumulated would probably gradually oxidize and disappear. It would even be possible that organic acidity might never accumulate under any condition of adequate aeration. But since soils are so variable in character and since widely different systems of managment are employed, no definite conclusions may be drawn.

CARBOHYDRATE MATERIALS

The mixture of degradation products of soil organic matter, which is too loosely classed as humus is perhaps somewhat influenced also in its reaction by carbohydrate materials. Though such materials may produce acids as acetic, propionic, and butyric, it is not known for how long a time they may exist unchanged. Natural albumin acids seem to disintegrate more slowly than the carbohydrate acids. Snyder of Minnesota (18) found that manure and other nitrogenous materials produced a humus of greater acidity than did straw and sawdust which contain less nitrogen. The fact that residues of a cellulose or starchy nature require more time for decomposition, makes possible a more complete oxidation of any acidity which might accompany the decomposition. In general, it seems that the most available carbon disappears from the soil rather more rapidly than does the nitrogen, so that, at times, it becomes a matter of importance to apply carbon-rich manures. The relative values of the different types of materials under the different systems of soil treatment is, however, a subject for further investigation.

PRESENTATION OF THE PROBLEM

It has been observed that very complex reactions are occurring continuously in the soil. These may be tending simultaneously toward acidity and basicity in soil reaction. Chemical potential demands that reactions shall occur in such a way as to result in the maximum entropy to a given system. As carbon dioxide is evolved from the soil or nitrogen is lost or used by plants or a hundred and one other reactions occur, there is a change in the chemical potential. Aside from the purely chemical, there are also what may be termed

life forces, including the action of any and all soil life. Even the enzymes of origin external to the soil may have an effect. For example glucosides contained in plant cells, are broken down by enzymes which are present in other cells from which they must be liberated before attacking the glucoside. The freeing of these enzymes and their activity upon plant residues may hasten availability of nutrients, both saccharine and non-saccharine in nature, to soil organisms. Physical factors in the soil may favor or disfavor certain of these ends and at times results appear in reverse order to expectations. Arbitrary statements should not be made, therefore, in regard to data from a study, which is at best, conducted under more or less unnatural conditions and burdened with many limitations.

It may be presumed that no material ever becomes of use as a fertilizer until some organism breaks it down into a simpler form. The changes are brought about by an immense number of organisms, the end products depending upon the nature of the substrate and the environment. Usually the more desirable products are favored by aerobic activity, but the conditions cannot always be regulated in either field or laboratory. It is probable, however, that for every molecule of ammonia split off there may be formed at the same time under any condition an equivalent of amount of acid, even though the molecule may exist only momentarily.

Whatever deleterious effects may be produced by such transitory acids are probably not worthy of consideration. When organic matter is added to soils there is not only an increased absolute amount of food, but reactions which occur lead to an increased variety of degradation compounds. Since every variety of substance, be it acid or base, is select food for a special organism or group of organisms, a more multitudinous and varied flora follows the addition of organic materials. Greater bacterial activity means increased available mineral food, due to the dissolving action of acids or carbon dioxide produced. This effect should be perceptible in the rate at which calcium carbonate added to the soil is decomposed. Consequently in these studies residual carbonates, as well as acidities, have been determined. The disappearance of carbonate and increase in acidity may not necessarily run parallel. In fact later data show that carbonates have practically all disappeared on treatments which have diminished the acidity, while there is yet a portion of the carbonate remaining on treatments which have increased the lime requirement. By combining acidity, carbonate and nitrogen changes a more intelligent interpretation of the various soil reactions may be reached.

PLAN OF EXPERIMENTS

Typical of highly nitrogenous materials, albumin, casein and blood have been studied. Starch and dextrose have been used as carbohydrates. And as materials of more practical significance alfalfa and ammonium sulfate have been employed. Two soils were tested with all treatments. The studies

were made in the greenhouse using gallon earthenware jars for containing vessels. Four samplings were made at intervals of 2, 5, 10 and 15 weeks, respectively, thereby covering a total period of a little more than 100 days. The results by White (20, 21) were reported for several months, a much longer period, as were those by Miller (13). Skinner and Beattie (17) made field studies covering a period of 5 years. In this study, however, rather easily decomposable materials were used and it is doubtful if more significant results might have been obtained from a longer study. Ordinarily crop residues might require a longer time for decomposition.

On each sampling 4 determinations were made, the ammonia, nitrates, acidity and carbonates of limed soils. On the second and last samplings Dr. Potter had planned to determine the soluble non-protein and perhaps other forms of nitrogen, but circumstances made it necessary to report the results incomplete as will be seen in the following paper by Potter and Snyder.

The soils were quite distinct in type. One was from the humus plots of the station, a dark soil fairly rich in organic matter, classified as Carrington silt loam. The other soil was from the Agronomy farm, rather sandy, light in color, and low in organic matter, probably classified as Carrington sandy loam.

Most of the organic treatments were made at the rate of 10 tons per acre. One application on the humus soil was made at double this rate for alfalfa and the ammonium sulphate was added in all cases at the rate of only one ton per acre.[2] It might be argued that these are excessive treatments, and that they would not apply to practical farm conditions. But when it is remembered that there is a more thorough mixing and that the materials not naturally existing in that condition are more finely ground than in field treatments, it would not seem likely that any physical effect produced by the treatment here would be greater than that occurring naturally in soils. Organic matter turned under in the field is likely to occur in a layer which is not well distributed and the concentration of material locally might be even greater than that prevailing in the pot treatments. It would not be maintained, however, that identical decompositions would occur.

Optimum moisture conditions were provided as nearly as possible by daily watering. Though pots cannot be controlled as accurately as tumblers, and checks are consequently not as good, the results are more nearly comparable with field conditions and of more value for that reason. Moisture and temperature fluctuations are perhaps greater in the field. These studies began in April when greenhouse temperatures were relatively low. In July and August on the other hand, extremes of 115° to 120°C. were reached. Moisture conditions were subject to similar changes. In very hot dry weather the surface soil of the pots became quite dry even with daily watering. In moist weather on the other hand, the soils never dried out and demanded only a little water

[2] All calculations are made on the air dry basis.

once or twice per week. These variable conditions, no doubt, had their effect upon the results obtained, but they may be regarded as indicative of what may occur in the field. There is not, however, the leaching and over saturation of water, or the effect of plant growth to which field treatments would be subject.

AMMONIFICATION[3]

The ammonia produced was determined by the aeration method using potassium carbonate to free the ammonia from its salts. The method has proved very accurate, as duplicates agree closely, even when corresponding determinations are run at different times. After the first sampling it was decided to run only one determination since differences due to the various treatments were usually quite marked and variations due to manipulation of the method were not significant. Though ammonification probably began in a few hours or at most in a few days, information on this phase of the question was not especially desired and determinations were not made except at the regular periods of samplings. The results are shown in table 1.

With albumin and casein it is observed that the greatest amount of ammonia was found at the first sampling on both soils. The chief cause for the large amounts of ammonia at first is the failure of nitrification to begin. It may be noted that there are no nitrates at the first sampling except in the casein-treated pots of the humus soil. The multiplication of organisms, however, may be inferred to have been occurring at this time. Consequently more and more of the changed materials is being resynthesized as the experiment progresses. It is notable too, that though casein seems to have nitrified more quickly than albumin, there is also a greater accumulation of ammonia, showing more rapid progress of both ammonia and nitrate production at first. After the first sampling the results are fluctuating. By far the largest absolute amount of ammonia is found in the humus soil, indicating a superior ammonifying power for this soil. With blood on the other hand the largest amount of ammonia is found at the last sampling in spite of the fact that there is greater nitrate production at the same time. This is illustrative in a quite definite way of the difference in ease with which different organic materials are decomposed.

The carbohydrate materials give such small amounts of ammonia that interpretation of the results is not significant. Since no nitrogen is added by starch or dextrose no great amount of ammonia could be expected. As a matter of fact the untreated soil produced practically the same quantity. Alfalfa and ammonium sulfate behave in much the same way. A little more nitrogen is added in the alfalfa but it is probably not quite as rapidly available. After the first sampling there is no more ammonia with alfalfa treated soil, than

[3] All results with ammonification and nitrification are expressed as parts of nitrogen per million parts of soil.

TABLE 1

Amount of ammonia at the end of each period

TREATMENT		1ST SAMPLE, 2 WEEKS		2ND SAMPLE, 2 WEEKS		3RD SAMPLE, 10 WEEKS		4TH SAMPLE, 15 WEEKS	
		No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
Humus soil		*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p..p.m.*	*p.p.m.*	*p.p.m.*
Soil alone.....	1	14	16.8	5.6	11.2	16.8	11.2	16.8	11.2
	2	16.8	21.0	11.2	22.4	5.6	16.8	16.8	16.8
Albumin.....	1	982.8	676.4	588.0	173.6	632.8	100.8	856.8	112.0
	2	917.0	705.2	666.4	168.0	800.8	72.8	588.0	78.4
Casein.....	1	917.0	855.3	515.2	448.0	582.4	56.0	627.0	39.2
	2	982.8	784.0	582.4	448.0	532.0	33.0	448.0	28.0
Starch.....	1	22.4	14.0	11.2	16.8	16.8	11.2	16.8	11.2
	2	21.0	7.0	16.8	16.8	16.8	16.8	16.8	11.2
Blood.....	1	350.0	299.6	352.8	448.0	526.4	140.0	548.8	100.8
	2	393.4	322.0	386.4	392.0	487.2	28.0	560.0	22.4
Dextrose.....	1	23.8	23.8	16.8	11.2	16.8	16.8	28.0	11.2
	2	23.8	14.0	16.8	16.8	28.0	16.8	28.0	11.2
Alfalfa.....	1	22.4	19.6	5.6	16.8	16.8	16.8	16.8	11.2
	2	19.6	16.8	16.8	16.8	5.6	22.4	11.2	11.2
Double alfalfa.	1	39.2	19.6	16.8	16.8	16.8	11.2	11.2	11.2
	2	30.8	22.4	16.8	16.8	16.8	16.8	16.8	16.8
Ammonium sulphate....	1	95.2	22.4	50.4	16.8	28.0	22.4	16.8	11.2
	2	98.0	14.0	50.4	5.6	22.4	16.8	16.8	11.2
Sandy soil									
Soil alone.....	1	11.2	11.2	11.2	11.2	11.2	16.8	16.8	11.2
	2	11.2	16.8	11.2	16.8	11.2	22.4	11.2	22.4
Albumin.....	1	660.8	546.0	616.0	324.2	571.2	509.6	560.0	313.6
	2	672.0	504.0	621.6	358.4	711.2	476.0	521.2	268.8
Casein.....	1	809.0	761.6	532.0	156.8	543.0	229.6	672.0	117.6
	2	786.8	772.3	588.0	285.6	616.0	207.2	599.2	44.8
Starch.....	1	22.4	16.8	11.2	11.2	11.2	11.2	16.8	22.4
	2	11.2	5.6	11.2	11.2	11.2	11.2	22.4	16.8
Blood.....	1	498.4	408.8	420.0	448.0	481.6	151.2	565.6	67.2
	2	518.0	408.8	408.8	392.0	481.6	212.8	565.6	140.0
Dextrose.....	1	11.2	11.2	11.2	11.2	16.8	16.8	22.4	5.6
	2	16.8	11.2	11.2	11.2	22.4	11.2	44.8	11.2
Alfalfa.....	1	39.2	33.6	11.2	11.2	11.2	5.6	11.2	11.2
	2	39.2	16.8	11.2	11.2	11.2	22.4	11.2	11.2
Ammonium sulfate.....	1	134.4	72.8	95.2	11 2	123.2	11.2	78.4	28.0
	2	140.0	89.6	95.2	5.6	112.0	16.8	72.8	16.8

with untreated, due to nitrification. The ammonium sulfate pots run a little higher, especially on the untreated sandy soil. This is due to lack of nitrification in the absence of lime, since the ammonia obtained in the determination represents the unchanged fraction of the ammonium sulfate. On the humus soil which gave a higher lime requirement, however, active nitrification occurred.

The effect of the lime in depressing ammonification is very characteristic, agreeing with results obtained by previous workers, where rather large amounts of lime were applied. (Six and seven tons, respectively, on the sandy and humus soils were used). Where the nitrogenous organic materials are added the depression is quite extreme. Part of the depression is only apparent, however, since nitrification is much greater in the presence of lime. But the summary table shows that there is yet a depression in nearly every case except where ammonium sulfate is used even when allowing for the change to the nitrate form. Still another factor may enter here. Organisms are usually more numerous in the presence of lime if soils are acid, and a greater nimber of organisms might have resulted in a greater amount of both nitrate and ammonia being synthesized into new proteins. The importance of this factor is questionable.

The nitrogen content of the materials used was 15.24 per cent, 14.25 percent, 14.48 per cent, and 2.92 per cent, respectively for the blood, casein, albumin, and alfalfa. Since this is adding nitrogen at the rate of 1524, 1425, 1448, and 292 parts per million of soil, it is easy to calculate the per cent which is changed to ammonia at any time. The casein and albumin approximate 50 to 60 per cent at the first sampling on the humus soil and 40 to 50 per cent on the sandy soil at the same time. Blood starts at about 20 per cent, and runs up to something like 30 per cent, on the humus soil but starts at better than 30 per cent, on the sandy soil and never runs much higher. Only a small per cent of the nitrogen of the alfalfa even exists in the ammonia form.

The general behavior of the two soils is noticeable because of characteristic differences. The sandy soil promotes a slower ammonification in practically every case except for blood. The effect of lime in depressing ammonification is apparently greater on the humus soil at the last two samplings. Previous to that time there is less difference.

The cause for greater ammonification in the absence of lime, aside from the differences due to change to nitrate, may be due to differences in the soil flora. In acid soils a large part of the ammonification may be due to fungi. This is in accordance with the conception that organic soils contain chiefly molds, rather than a bacterial flora. Though these soils are not especially humus-rich, a rather heavy application of organic material has been made, and the acidity by depressing bacteria, would tend to stimulate mold growth. Though molds may be abundant even under conditions which furnish an alkaline reaction, bacteria do not thrive in acidity, and there is, therefore, less competition to the mold organisms. Contributory to this thought McLean and Wilson

(14) of New Jersey found that moulds were very efficient ammonifiers, converting nearly half the nitrogen of dried blood in 7 days. It is known, also, that ammonification occurs in flooded soils on which rice is grown where mold growth and anaerobic bacteria would be responsible for the change.

A preliminary test with tumblers has shown that molds are very abundant in these soils made artificially acid with sulfuric acid and treated with 10 tons of albumin. The greater the acidity the greater the mold growth has been up to a certain limit at least. The normal soil showed little mold growth, and in fact, there was no great amount of visible mycelium or fruiting bodies until an acidity equivalent to 7.2 tons including that already in the soil was supplied. The 8.5 and 9.6 ton acidities developed likewise a heavy growth. Later just as heavy a growth appeared on the 17.5 ton requirement. Soils made acid without the addition of organic matter did not develop a heavy visible growth, showing that a combination of the two factors, acidity and

TABLE 2

A test in tumblers treated with H_2SO_4 after 5 weeks incubation, humus soil only

	ACIDITY	NITROGEN AS AMMONIA	NITROGEN AS NITRATES	SUM OF NITRATES AND AMMONIA
	tons	*p.p.m.*	*p.p.m.*	*p.p.m.*
Soil, albumin	3.7	700.0	255.1	955.1
Limed soil, albumin	2.7	453.2	416.6	869.8
Soil, albumin, acid	4.3	761.6	184.5	946.1
Soil, albumin, acid	6.1	770.0	143.7	913.7
Soil, albumin, acid	7.2	820.4	71.2	891.6
Soil, albumin, acid	8.5	795.2	53.4	848.6
Soil, albumin, acid	9.6	828.8	32.4	861.2
Soil, albumin, acid	17.5	728.0	Trace	728.0

organic matter was most favorable to mold production. In every case high acidity has favored molds as would be expected. The object in making the heavy application of acid was to intensify differences. These conditions make it possible to determine in a limited way the activity of the mold flora of the soil, and the results should suggest the capacity of molds for taking part in the nitrogen changes even under normal conditions. Of course, acid resistant bacteria might also be of significance. It is possible that the acid may denature the albumin by forming acid albumin yet the effect in this respect may not be vastly different from that which occurs in soils without the artificial acidity. More definite results from this study are reported below.

The addition of the albumin shows the same depressing effect upon the lime requirement of the normal soil. Sulfuric acid was chosen to increase the acidity because it is a strong mineral acid, and the sulfate ion would probably not have a deleterious effect on the organisms. There were duplicate tumblers but the checks agreed so well that only averages are presented in the table.

There is the same depression of ammonia and increase of nitrates in the presence of lime (the treatments were the same as in the original experiment except for the H_2SO_4). The absolute amount of ammonia is found to increase with increasing acidity fairly regularly up to the heaviest acid treatment. Even here there is only a slight depression. On the other hand the nitrates decrease quite regularly with increasing acidity until there are none or only a slight trace in the most acid treatment. Taking the sum of nitrate and ammonia nitrogen, the maximum is found in the normally acid soil and the next highest amount at the next acidity and on down until there is a lime requirement of 8.5 tons before there is a depression. The indication is, therefore, that ammonification continues under rather extreme acid conditions, while nitrification is much depressed and finally stopped by the same conditions.

Correlating these results with the very heavy gray and yellow mold grown in the tumblers it would seem quite probable that the ammonification was due to considerable extent to the molds. Yellow fruiting bodies were very abundant and the moldy odor was quite conspicuous. In the pot treatments, also, a rather conspicuous growth of a lead gray mold was observed. The evidence is at least in favor of the activity of molds as accounting for the greater ammonification under acid conditions rather than in the presence of lime.

A weak point in the study is of course the incapacity of the method used for determining hydrogen-ion concentration. But since sulfuric acid is a strong acid and enough was present actively so that an acid extract was obtained from the treated soils, it may be presumed that a higher concentration of acidity prevailed than is present in normally acid soils. The water extract showed increased acidity with increase in acid applied so far as the color of methyl orange could indicate.

Some discussion as to the significance of ammonia and other forms of nitrogen aside from the nitrate, in the nutrition of plants may be of indirect relation to this study. New conceptions are formed as the nitrogen problem is better understood. It has been suggested (10) that since the plant must reduce nitrate to ammonia in order to synthesize protein a saving of energy would result if a more complex form of nitrogen than ammonia such as an amino acid were used by the plant. Complying with this idea investigators have made tests, and a number of compounds, including arginine, histidine, xanthine, hypoxanthine, guanine, creatine, choline and nucleic acids, have been found to be directly beneficial to plants. Some of the materials have given results indicating that they were a better source of nitrogen than is nitrate itself. Rice which grows in the absence of nitrate uses ammonia and does not thrive in the presence of nitrate.

Along the same line Lipman (12) suggests the possibility of a diffusable compound passing from the roots of legumes and stimulating the growth of a non-legume. Such a postulation seems entirely reasonable in the light of the knowledge that such compounds as urea, glycocoll, asparagine, and lucine,

serve as sources of nitrogen for plant growth. It would not, therefore, be necessary that these compounds which are soluble and diffusible be nitrified before they could be of use to the non-legume. These same substances are also products of both analysis and synthesis by organisms, which not only makes possible beneficial effects of many organic manures as a source of nitrogen before nitrification occurs, but makes it likely that some benefits may accrue even in the absence of an efficient nitrifying flora. Compounds of greater complexity than dipeptids are relatively insoluble and beneficial effects due to their presence could hardly be expected. There is a slight possibility also that the beneficial effects sometimes attributed to micorrhizal growths may be due to partially synthesized nitrogen compounds of no greater complexity than dipeptids, which the host plant is able to absorb from the micorrhizal filaments. According to Coville (3) the fungi themselves habitually use organic nitrogen which they may obtain as well from acid soils. If then, as he also suggests they supply the host plant with some sort of nitrogen they become of double value, by supplying the nitrogen and likewise by saving energy to the host plant.

In the future it would seem at least justifyable to refrain from the declaration that plants must have nitrates. The solubility and nontoxicity of nitrate and the reaction products makes it an especially suitable plant food. Yet it is observed that when plants, as in the case of rice, fail to develop the necessary reducing enzyme to make use of nitrate in protein synthesis, there is no loss in thrift or efficiency of the plant organism.

And again when it is observed that although molds may not nitrify, they do produce ammonia, amino acids and dipeptides which are probably of direct use to plants, their activity has greater significance. The fact that more productive soils usually permit greater nitrification may be more incidental than fundamental. A well aerated soil is essential to productiveness but it likewise stimulates the production of nitrates. Under such conditions protein degradation products other than nitrates perhaps have little opportunity to function in the nutrition of plants. And while it is a fact that plants use nitrates it is also a pertinent question as Lathop (10) has suggested, to ask why it should be necessary.

It is not desired, however, to minimize the importance of the nitrifying process in soils. It is only by those oxidation processes that toxic substances are prevented from accumulating and such oxidations insure, thereby, the recovery of both carbon and nitrogen to serve again a cycle of usefulness.

NITRIFICATION

For the determination of nitrates the phenol-disulfonic acid method, as modified by Davis (5), in this laboratory, was used. The method has the advantage of accuracy and ease of adjustment to the quantity of nitrates by the making of proper dilutions. There is also the advantage of detecting small amounts of nitrate. The results are shown in table 3.

TABLE 3

Nitrates at each successive sampling

	2 WEEKS		5 WEEKS		10 WEEKS		15 WEEKS	
	No lime	Lime	No lime	Lime.	No lime	Lime	No lime	Lime
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Humus soil								
Soil alone	17.3	26.3	40.2	68.6	55.9	126.6	43.3	175.8
	17.7	26.3	40.4	93.7	62.4	187.3	87.6	215.6
Albumin	Trace	Trace	159.0	150.0	189.6	413.1	448.4	936.6
	Trace	Trace	223.2	236.2	275.6	589.5	451.3	664.6
Casein	67.0	26.0	395.5	620.9	524.4	789.6	468.7	1530.0
	80.6	26.0	468.7	427.8	414.3	774.6	684.7	1360.5
Starch	0.0	00.0	0.0	0.0	Trace	Trace	35.8	124.2
	0.0	00.0	0.0	0.0	70.5	77.1	89.4	185.4
Blood	83.4	157.0	375.5	485.7	291.6	429.5	492.2	542.8
	82.2	165.2	287.6	564.8	443.5	559.7	327.7	1345.0
Dextrose	Trace	Trace	23.5	34.5	53.2	73.6	91.1	133.2
	Trace	Trace	14.3	41.3	48.3	83.2	71.7	124.9
Alfalfa	58.4	65.7	138.4	135.5	157.8	150.1	318.0	259.8
	45.7	55.2	118.7	154.2	183.3	177.6	337.9	269.8
Double alfalfa	83.1	87.7	188.6	150.1	255.7	421.2	513.5	515.0
	58.7	80.4	179.5	159.2	300.3	310.2	180.6	324.1
Ammonium sulfate	40.7	123.2	109.5	197.8	95.5	317.4	138.7	302.0
	52.8	119.3	127.7	202.0	155.5	255.2	176.8	298.6
Sandy soil								
Soil alone	10.9	19.0	9.1	17.4	28.4	49.3	38.3	51.4
	10.9	19.0	10.4	16.4	28.4	47.7	36.7	41.8
Albumin	Trace	Trace	13.4	52.0	184.5	103.4	342.0	512.5
	Trace	Trace	14.0	13.0	97.5	118.5	241.4	558.6
Casein	Trace	Trace	30.0	42.0	149.8	353.9	408.1	645.3
	Trace	Trace	23.0	30.0	178.1	599.5	264.4	430.6
Starch	Trace	Trace	Trace	Trace	Trace	Trace	Trace	29.6
	Trace	Trace	Trace	Trace	Trace	Trace	Trace	44.1
Blood	31.6	41.4	351.9	425.1	314.6	560.9	676.9	717.6
	36.8	36.3	279.0	425.0	231.8	424.9	358.8	565.7
Dextrose	Trace	Trace	Trace	Trace	Trace	Trace	22.9	52.5
	Trace	Trace	Trace	Trace	Trace	Trace	26.1	46.5
Alfalfa	17.5	22.0	70.4	83.8	87.4	114.7	196.1	246.8
	26.9	21.4	72.1	96.2	112.5	118.9	202.1	176.8
Ammonium sulfate	22.6	56.9	58.5	192.5	74.2	128.9	206.1	374.0
	22.7	45.3	63.1	167.4	89.5	179.3	133.6	370.0

It is noticeable that the highly nitrogenous materials, albumin on the humus soil and casein likewise on the sandy soil, failed to show nitrates at the first sampling. This is probably due to a slight toxicity caused by the accumulation of ammonia at first. Then the rapid multiplication of organisms resulted in the exhaustion of the original soil nitrates before active nitrification had time to begin. Nitrification in practically all cases is found to increase to the last sampling. Casein nitrifies most rapidly of any of the nitrogenous materials, on the humus soil, but on the other soil blood takes the lead. Blood also begins nitrification at once on both soils apparently because ammonification did not begin at such a rate as to render its accumulation toxic. Consistent with its character in ammonification, blood nitrifies gradually, and finally produces the maximum absolute amount of nitrate in the sandy soil. In the humus soil, casein holds this place, though one pot in the last sampling of blood was a close second. With the exception of this one determination, blood is second to albumin on the humus soil. In contrast to the rapid nitrification of blood, albumin fails to nitrify at a comparable rate until the last sampling on the sandy soil, and not until the third sampling on the humus soil. Casein on the other hand catches up with the blood at the second sampling on the humus soil, and somewhat at the third on the sandy soil.

Starch and cellulose behave in much the same way on both soils since nitrates disappear almost entirely and continue absent nearly to the end of the experiment. There are two explanations for this action. Either denitrification may occur in the presence of easily available sources of energy, or nitrification never sets in for the same reason. It is probable that the organisms do not nitrify except to the extent of satisfying the nitrogen demands of the soil flora. Such results would suggest a means for control of the rate of nitrification. In fact Kelly (9) has reported results showing a reduction of 50 per cent or more in the rate of ammonification of casein and blood, in the presence of starch. Brown and Kellog (1) obtained a similar depression of the oxidizing power of organisms in sulfofication studies. They found that cane sugar, starch, and filter paper all depressed the oxidation of the sulfur, the greater the amount and the solubility, the greater the depression. Gerlach and Densch (8) found that easily decomposable organic compounds such as dextrose or straw caused soluble nitrogen salts such as ammonium sulfate and sodium nitrate to be converted into protein compounds in the soil. Later, however, these were readily decomposed. Similar results were obtained also in work done at New Jersey. Lipman, et al. (11) has suggested the possibility that the large evolution of carbon dioxide in the presence of carbohydrate material might account for the non-appearance of nitrates. Coleman (2), however, found that a smaller amount of dextrose (0.5 per cent) increased the rate of nitrification. This increase may be attributed to increased numbers of organisms which soon exhaust the dextrose and then attack the nitrogenous organic matter quite vigorously. Brown suggests a temporary

toxicity to the nitrifying flora as a possible cause of non-appearance of nitrates. The results are illustrative of the inter-relationships of carbohydrate and protein organic portions of the soil, and suggest a problem for study to determine more definitely what the relationship may be. It might be possible to exercise a measure of control over nitrogen changes by making proper protein and carbohydrate combinations.

Wright (22) in making a study of various organic materials upon nitrification concluded that coarse material plowed under in the undecayed state, may materially reduce the quantity of available nitrogen in the soil. Plowing under such materials in the fall should, therefore, save nitrogen from loss during the winter and by spring permit nitrification sufficient for crop production. This author found, however, that green and succulent material was rather easily and quickly attacked. Evidently there are several factors which may determine the susceptibility to nitrification and, therefore, the more advisable time and rate of application of an organic fertilizer.

In line with the results it may be inferred that nitrification in excess of nutritional demands begins when easily available carbohydrate is exhausted. Nitrification starts in every case to an appreciable extent, except on the unlimed starch-treated sandy soil. Nitrification begins soonest on both soils in the presence of dextrose, probably because dextrose is more easily available and is, therefore, more quickly exhausted. The insolubility of the starch and the protective covering of cellulose about the starch would render it quite resistant, and probably result in a large residue of unavailable material. The limed treatments start nitrification first and produce greater quantities of nitrates, which is also consistent, since more organisms should be active in the presence of lime, and carbohydrate would, therefore, disappear more rapidly. The argument applies again consistently in that in the humus soil which should possess a more multitudinous flora to exhaust the carbohydrate, nitrification begins first in the presence of both starch and sugar. Evidence, therefore, points to lack of nitrification rather than to denitrification as the cause of absence of nitrates.

Doryland (6) presents results which are in line with the above conclusions and in his publication may be found also an excellent historical review and bibliography. The results of previous workers in general, justify the interpretation presented in this paper.

Alfalfa behaves in much the same way as blood, gradually increasing in absolute amount of nitrates up to the last sampling. It is notable, however, that there is not twice as much nitrate produced when the rate of adding alfalfa to the soil is doubled. In only a few cases is it nearly double. Ammonium sulfate makes very consistent and gradual increases up to the last sampling.

The difference in the behavior of the two soils is shown more characteristically in nitrate production than in other respects. The humus soil has a much greater nitrifying power, in respect to changing both its own organic

matter and that added to it. Probably a part of the variation is due to difference in soil flora and this in turn is due to lack of organic material in the original sandy soil. And too, if it is only in the soil films about the soil particles that organisms are active, as is probable, the greater number of particles in the humus soil would mean greater film space in which activities might occur. According to data at hand the humus soil should have a surface area of its particles just about twice that of the sandy soil, or equivalent to $2\frac{2}{3}$ and $1\frac{1}{3}$ acres of surface respectively. This alone is sufficient to account for the difference in activity of organisms in the two soils, though the amount of bacterial food may be an important factor as well.

The stimulation of nitrification due to the presence of lime is too conspicuous to need pointing out. There are, however, a few exceptions. Ammonium sulfate, which is physiologically acid, and casein, which is rather more acid than basic, respond quite strongly. Blood and albumin which are not predominantly acidic do not make so consistent a response to lime. And alfalfa which may be considered physiologically basic, due to the fact that upon complete oxidation a basis residue remains, responds still less. On the other hand the humus soil which is more acid by a ton and a half is much the more active soil. The indications are, therefore, that other factors may be of greater weight than is soil acidity, at least when this acidity is measured only in terms of total reactive acids present. Along with this may be taken the fact that a maximum nitrification is reached at the last sampling, which indicates that materials other than carbonates were functioning as a base, as the carbonates have been practically exhausted long before in the cases where nitrification is greatest.

Acidity is probably overemphasized sometimes, at least to the extent that lime is given too much credit from this standpoint. It is entirely probable that a neutral soil might respond to lime in a nitrification study due partly to the indirect effect of the carbonate on the soil flora or for other reasons. Aside from its neutralizing power, an important effect upon the soil flora comes indirectly through the influence of lime in improving the mechanical condition of the soil.

NITROGEN SUMMARY

In table 4 a summary of the nitrogen relationships is presented by taking averages and adding the ammonia and nitrate together. There is also given an average of all samplings with and without lime, showing the results after deducting the soil itself, which may be considered as a blank.

It is quite remarkable that on both soils the sum of the nitrates and ammonia is greater in most cases on the unlimed than on the limed pots where organic treatments are given, but that the reverse is true where there is no addition of organic material. These results would indicate that lime could act toward conserving the organic matter at least when fairly heavy applications were made. The ammonium sulfate-treated soils behave as the soil alone, show-

TABLE 4

Nitrogen summary. Sum of ammonia and nitrates

	2 WEEKS		5 WEEKS		10 WEEKS		15 WEEKS		AVERAGE			
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime	No lime		Lime	
Humus soil	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*		*p.p.m.*
Soil alone	32.9	45.2	48.7	97.9	70.3	170.9	82.2	209.7	58.5	minus soil	130.9	minus soil.
Albumin	949.9	690.8	818.3	363.9	949.4	588.1	1172.2	895.8	972.4	913.9	634.6	503.7
Casein	1023.7	845.6	980.9	972.3	1026.5	826.6	1114.2	1478.8	1036.3	977.8	1030.8	899.9
Starch	21.7	10.5	14.0	16.8	52.0	52.5	79.4	166.0	41.8	−16.7	61.4	−69.5
Blood	454.8	471.9	701.1	945.2	874.3	578.6	964.3	1005.5	748.6	690.1	750.3	619.4
Dextrose	23.8	18.9	35.7	51.9	73.1	95.2	109.4	140.2	60.5	2.0	76.5	−54.4
Alfalfa	73.1	78.6	139.7	161.6	181.7	183.4	341.9	276.0	184.1	125.6	174.9	44.0
Double alfalfa	105.9	105.1	200.8	171.4	294.8	379.7	361.1	433.5	240.6	182.1	272.4	141.5
Ammonium sulfate	143.3	139.4	169.0	211.1	150.7	305.9	174.5	311.5	159.4	100.9	242.0	111.1
Sandy soil												
Soil alone	22.1	33.0	20.9	30.9	39.6	68.1	51.5	63.4	33.5		48.8	
Albumin	666.4	525.0	632.5	373.8	782.2	603.7	832.3	826.7	728.4	694.9	582.3	533.5
Casein	797.9	766.9	586.1	257.2	743.4	695.1	971.8	619.1	774.8	741.3	584.6	535.8
Starch	16.8	11.2	11.2	11.2	11.2	11.2	19.6	56.4	14.7	−18.8	22.5	−26.3
Blood	542.4	447.6	729.6	845.0	754.8	674.9	1033.4	745.2	777.5	744.0	678.2	629.4
Dextrose	14.0	11.2	11.2	11.2	19.6	14.0	58.1	57.9	25.7	−7.8	23.6	−25.2
Alfalfa	61.4	46.9	82.4	101.2	111.1	130.8	210.3	223.0	116.3	82.8	125.5	76.7
Ammonium sulfate	159.8	132.3	156.0	188.3	199.4	168.1	245.4	394.4	190.1	156.6	220.8	172.0

ing an increase of total nitrogen changed, due to the application of lime. Different results would probably be obtained under field conditions, since in practically all tests that have been reported lime has resulted in a more rapid depletion of organic matter.

The greater increase in nitrogen change on the untreated soil emphasizes the need of keeping up the organic supply of nitrogen-poor soils. The rate of change is more than doubled in many cases by the presence of the lime, which means that poor soils would be soon exhausted under such treatment. Though the absolute amount of nitrogen changed over is much greater, due to the added organic materials, the rate of change is more economical, and if conditions may be provided such that either plants or the soil organisms can take up the nitrate as produced, there is no loss. The use of cover crops would assist in securing this end.

The sandy soil seems to have converted the nitrogen of the blood somewhat more efficiently than did the humus soil. The same can be said of the ammonium sulfate on the unlimed pots. The total nitrogen changed is observed to have been greater at the first sampling than at the two succeeding ones for the albumin and casein, and then to have increased again at the last sampling. These results are probably due to the multiplication of organisms. Later the protein of their bodies was changed over again. It is, therefore, impossible to determine the proportion of total original material that may have been changed. Some doubtless passed through the cycle of changes 2 or 3 times while very resistant residues remain unattacked. As to whether the synthesized proteins of bacterial cells might be more susceptible of nitrification, there is little evidence. Claims are made both ways. Probably the correct interpretation is that some bacterial proteins are more easily changed than some original soil protein and vice versa.

Taking average figures it will be noted that about a third of the albumin nitrogen, more than half the casein nitrogen, somewhat more than a third of the blood nitrogen, less than a tenth of the alfalfa nitrogen and nearly two-thirds of the ammonium sulfate nitrogen has existed in the form of ammonia and nitrates on the humus soil in the presence of lime. Without lime more than 60 per cent of the nitrogen, less than half the nitrogen of blood, a similar proportion of the alfalfa nitrogen, and about half the ammonium sulfate nitrogen has existed in these forms. Considering maximum figures, nearly 80 per cent of the albumin, nearly 90 per cent of the casein, a little more than half the ammonium sulfate nitrogen has been changed in the humus soil. In some cases the maximum changes occur on the limed, and in others on the unlimed pots. The figures for the sandy soil would run considerably lower in most cases.

An attempt with little success was made to correlate the composition of the protein with its rate of nitrification. A difficulty lies in the fact that the composition does not tell the manner in which the different amino acids are linked together in the protein molecule. This is probably as great a factor as is the nature of the actual amino acids present.

Blood is probably over half hemoglobin, nearly a third albumin, and contains other constituents. The albumin has been studied but there is no data as to the decomposition of hemoglobin or other portions of its constituents. The gradual and regular decomposition of blood may be accounted for because of the fact that it is a mixture of proteins, some portions probably decomposing slowly and thereby tending to hold the absolute activity in check. It is known that hemoglobin resists the activity of putrifying organisms, but whether due to anti-ferments, or for other reasons is not known. The slow decomposition of the blood probably is due in part at least to the hemoglobin present.

ACIDITY RESULTS

The acidity results are summarized in table 5. The modified Tacke Method (19) as previously described, was used. Briefly this method consists in bringing the acid soil into contact with pure calcium carbonate, using water as the contact medium. After thorough shaking and aeration for 10 hours the carbon dioxide liberated by the acid soil is titrated and the result is considered the lime requirement of the soil. The data is presented in terms of tons of calcium carbonate per acre.

TABLE 5

Lime requirement of the variously treated soils, in tons per 2,000,000 pounds of soil

	2 WEEKS	5 WEEKS	10 WEEKS	15 WEEKS	MORE OR LESS THAN THE SOIL ALONE			
					1	2	3	4
Humus soil	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*
Soil alone	4.60	4.10	4.10	4.00				
Albium	2.16	3.35	2.60	2.85	−2.44	−0.75	−1.50	−1.15
Casein	2.45	3.95	3.95	3.90	−2.15	−0.15	−0.15	−0.10
Starch	4.55	3.85	4.10	3.55	−0.05	−0.25	0.00	−0.45
Blood	3.85	3.90	4.00	3.70	−0.75	−0.20	−0.10	−0.30
Dextrose	3.85	3.95	4.45	3.95	−0.75	−0.15	+0.35	−0.50
Alfalfa	4.10	3.95	4.30	4.05	−0.50	−0.15	+0.20	+0.05
Double alfalfa	4.10	4.00	4.15	4.50	−0.50	−0.10	+0.05	+0.50
Ammonium sulfate	4.80	4.75	5.10	5.45	+0.20	+0.65	+1.00	+1.45
Sandy soil								
Soil alone	3.05	2.50	2.60	2.65				
Albumin	1.30	1.25	1.35	2.40	−1.75	−1.25	−1.25	−0.25
Casein	1.50	2.40	1.65	2.15	−1.55	−0.10	−0.95	−0.50
Starch	2.60	2.50	2.15	2.55	−0.45	0.00	−0.45	−0.10
Blood	1.95	2.85	2.30	2.85	−1.10	+0.35	−0.30	+0.20
Dextrose	2.85	2.55	2.25	2.95	−0.20	+0.05	−0.35	+0.30
Alfalfa	2.80	2.70	2.25	2.40	−0.25	+0.20	−0.35	−0.25
Ammonium sulfate	2.95	3.15	3.25	3.25	−0.10	+0.65	+0.65	+0.60

The albumin and the casein have acted very much as an application of lime at first. This could be explained as due partly to the rapid production of

ammonia without accompanying nitrification to increase acidity. The albumin fluctuates (partly perhaps due to errors of sampling) more than does the casein, but causes greater reduction in lime requirement at the first sampling, and much greater reduction in succeeding samplings. This is not in accordance with the predominantly acidic nature of casein, and indicates decarboxylation or oxidation of acidity. Starch and dextrose have likewise, though to a smaller extent, reduced the acidity, except at the third sampling on the humus soil. On the sandy soil both carbohydrates have had a very fluctuating effect, and could scarcely be given a preponderance of credit either way. It is probable that organic acids may have oxidized faster in the presence of the dextrose and starch, and there is also the fact that nitrification does not occur to increase acidity. Blood, like the other protein materials, reduces the acidity considerably at first, but after that only to a slight degree on the humus soil, and on the sandy soil its conduct is not consistent either way. Alfalfa has first reduced the acidity and then increased it on both soils, but only to such an extent as to be insignificant. Its very complete nitrification would have the effect of increasing acidity, while the basic residue of minerals would have the opposite effect. The ammonium sulfate has caused a gradual increase in acidity on both soils, though to a less extent on the sandy soil. The data shows it to be the only material which could be definitely stated to have increased the acid reaction of the soil, excluding nitric acid itself, which is rather strong evidence that mineral acids are much more likely to act deleteriously. Several of the other compounds produce much more nitric acid, and any of them are potentially capable of producing organic acids, but since no other treatment has an appreciable sulfuric acid residue, the major effect must be attributed to this.

ACIDITY RELATIONSHIPS

The relation between soil acidity and the activity of soil flora is a somewhat complicated problem. As the soil reaction changes, group relations of soil organisms must shift. This is especially true since a high concentration of hydrogen-ion which is the true acidity, is deleterious to many bacteria, such as the nitrate producers, and azofiers but is beneficial to certain fungi, more especially the molds.

There is also undoubtedly a marked adaptation of the flora to soil reaction. Acidity must naturally result through a cumulative process, and as the intensity increases a more and more acid resistant strain of organisms of any species would seem natural. Such an adaptation would be comparable to that purposely brought about in the preparation of vaccines by the producing of strains of pathogenic organisms of weak virulence. Not only are the physiological, but likewise the morphological characters, very much subject to modification. Though there has never been a demonstration to the effect that a more efficient strain of organisms, such as the nitrifiers, may be pro-

duced, the cause may be attributed largely to the lack of methods. In fact, the organisms may be less efficient, but more resistant to acidity. Accurate methods, unless it be the hydrogen electrode method little used in soil studies, are not available for determining the active acidity of the soil. The ideal plan for such a study would be to start with a neutral soil, and by the use of artificial acids to prepare soils of definite hydrogen-ion concentration increasing by succeeding degrees to a high intensity. Then a study, where both pure and mixed strains of organisms were used should detect any adaptation, which might occur through perhaps several months time.

From its effect upon the activity of organisms, the question may be asked, how does acidity occur in the soil? It is seldom, at least, that a titrable acidity can be extracted from soils, yet the same soils turn litmus paper, and give other indications of a functioning acidity. Acids are not active until ionized and not ionized until dissolved. Therefore, there must be a soluble acidity, even though it cannot be extracted. Buffering would, of course, explain the fact that a soil extract is neutral to indicators. In fact, by mixing mono- and di-sodium phosphates a neutral solution may be prepared, which would require the addition of 0.5 equivalent of HCl or NaOH before the reaction would be acid to congo red, or alkaline to phenolphthalein. This buffer action may be brought about by various acid salts, amphoteric substances, salts of weak acids and bases, etc., and undoubtedly some soils possess a large reserve acidity or alkalinity. But it seems also that it is not possible to extract a buffered solution from the soil (a number of tests were made on these treated soils) as indicated by incapacity of the extract to decompose calcium carbonate. Yet both artificially prepared buffered solutions and acid soils decompose carbonate easily. It would be of interest to know whether an oil pressure extract from acid soils might not possess a buffer action.

That many soil acids, including those of both organic and mineral nature, are quite insoluble and inactive for that reason is likewise quite without question. Taking all of these facts together it is easy to see that the absolute acidity of a soil may not have a very definite significance, since it is measured by the total amount of base taken up, and gives no indication as to what portion of the measured value may be due to reserve acidity and relatively insoluble acids. The reserve acidity is probably of little or no direct injury to either plants or organisms, though it is likely to use up the carbonates applied to the soil.

The evidence taken together is strong, therefore, for a soluble acidity, but just how and where it exists is not so easy to say. From the different reactions given the conclusion that such soluble or active acids as are present exist only in the soil film, would not be irrational. It is probable that only the film water functions in the nutrition of plants or is active in promoting the activity of soil organism. It is consistent with the nature of protein substances and organic matter in the humified state that they should form a colloidal film about the mineral particles of the soil, binding the water

as a colloidal hydrate. The force which holds this film is difficult to measure, but it has been estimated at from 6,000 to 25,000 atmospheres. If organisms are active, principally in this film, and plant roots feed from the same film water, then there is an intimate relationship, on the one hand, between the reaction of the soil, which may perhaps not extend its sphere beyond the film thickness, and bacterial activity, and on the other hand between soil reaction and crop production. Such intimate relationships make it possible for the growing crop to exert considerable influence upon the changes brought about by the organisms and vice versa. Root hairs cling to soil particles, and organisms likewise are separated from the particles only with considerable effort, which is more evidence of a closely existing relationship such as has been described. These are not essentially new ideas but are a somewhat modified interpretation. Soil acidity, though without adequate reason perhaps, has been attributed to absorption forces, be they chemical or physical, which are probably somewhat similar to the force which binds water in colloids and which holds the films about particles. Nevertheless acidity to the degree that it is soluble may exist in the soil film and yet not be an adsorption phenomenon as popularly interpreted. It would not be presumed that all acidity or all colloidal matter would exist in films, though the active portion might be limited to such a sphere of functioning. Any which long existed in the free state would naturally be washed from the soil in the drainage.

Plummer (15), by the use of the hydrogen electrode, decided that soils vary appreciably in the hydrogen-ion concentration of the soil suspension. Similar results were obtained by Sharp and Hoagland (16). Plummer (15) worked also on the soil film water, which was obtained by Morgan's oil pressure method. It is significant that he found the reaction of this film water to be qualitatively the same as that of a soil suspension. The concentration of acidity or alkalinity was much greater, however, in the film water. The general conclusion of these workers is that an acid soil must always present an acid solution to its plants.

Another point of interest in the interpretation of these studies is the effect of the nitrification process upon the soil reaction. The nitric acid produced makes heavy drains upon carbonates, as the data shows, the amount of base used being somewhat, if not entirely, proportional to the nitrate. At the same time not only the ammonia but other nitrogen bases,[4] including the various amines, choline, guanidine, purine, and pyrimidine bases, etc., serve in the same way. Some nitrogen bases are stronger even than sodium hydroxide, and though no definite measures can be made, the organic acids produced, may not be any more significant than are bases liberated. The

[4] A nitrogen base results whenever a compound possesses nitrogen which may change in valence from 3 to 5. In water solution this change may result in the formation of an hydroxide as in the case of ammonia. In other cases the nitrogenous compound may be presumed to combine directly with the acid. If it were hydrochloric acid, an hydrochloride would be formed.

data in a general way confirms this idea, though a basic material would probably be nitrified under conditions where one predominantly acid would not.

In fact Funchess (7) of Alabama found that certain nitrogen bases, such as quinoline and guanidine carbonate, were nitrified best in acid soils. The effect was marked to the degree that lime prevented the decomposition of such materials. Other nitrogen bases, however, nitrified in the presence of lime, some more rapidly than in acid soil.

Another point related to the interpretation of the data is the effect of the original materials on the soil reaction. Since proteins have a reserve acidity, they might be expected to decompose calcium carbonate. But the addition of the same amount of proteins as was added to the soil in the studies produced no measurable effect upon the lime requirement. This may be explained if in the original protein material there is an internal salt formation, due to the free carboxyls neutralizing the free amino groups. It is thought that such a ring formation does exist. It would seem, therefore, that some chemical or enzymatic action were necessary to break the ring formation and start decomposition before a measurable effect is produced on the soil reaction. The data indicates that such changes occur rather rapidly, however, as maximum effects are produced at the first sampling.

RESIDUAL CARBONATES

The residual carbonates were determined with the same apparatus used to determine the acidity, using phosphoric acid (strength 1 to 15) to decompose the carbonate. Blanks were always run on the untreated soil and subtracted from the results. The blank is nearly always low, about 1 cc. and did not differ greatly on the variously treated pots. The results are shown in table 6 expressed in tons per acre.

The data show again that albumin, casein, and likewise to a lesser degree, blood show a protective action for the lime at the first sampling. Since these materials act as a base in both acidity and carbonate determinations, as is evident, it would seem that decarboxylation must have occured rather rapidly. This would of course always leave a basic residue. Doubtless too, the ammonia again would tend to preserve mineral bases of the soils. These conclusions are further justified from the fact that though casein is naturally predominantly acidic, it renders greater protective action than any of the other treatments. This again would indicate that much organic acidity might be rather ephemeral than significant. After the first sampling the drain upon the carbonates with these same treatments becomes marked. This is apparently due to the fact that nitrification has begun, thereby not only removing the basic action of the ammonia, but increasing the acidity by producing nitric acid. The changing of 28 pounds of ammonia nitrogen to the nitric form may theoretically increase the lime requirement by 200 pounds, due to the double action of removing base and producing acidity. This alone might

account for a considerable change of reaction. Blood and casein produce somewhat more violent effects in using up carbonate than does the albumin. On the sandy soil the blood produces very strong effects. Starch and dextrose have not caused marked effects, but have had a tendency to use up carbonates. It will be remembered that they had a tendency also to decrease acidity, so that taken together, their application is without significance in effect upon soil reaction. At the third sampling dextrose on the sandy soil has caused a marked drain upon carbonates but the high figures are probably due to error. The alfalfa treatment, likewise, has had no consistent effect upon the supply of carbonates, which might be predicted from its nature.

TABLE 6

Residual carbonates on treated soils at the several samplings

Expressed in tons per acre

	1	2	3	4	MORE OR LESS THAN SOIL UNTREATED			
					1	2	3	4
Humus soil	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*	*tons*
Soil alone	2.75	2.50	1.65	1.90				
Albumin	4.65	1.00	0.90	0.00	+1.90	−1.50	−0.75	−1.90
Casein	6.10	0.50	0.65	0.05	+3.35	−2.00	−1.00	−1.85
Starch	2.65	2.00	2.45	1.80	−0.10	−0.50	+0.80	−0.10
Blood	3.45	0.75	0.55	0.15	+0.70	−1.75	−1.10	−1.75
Dextrose	3.15	2.15	1.60	1.55	+0.40	−0.35	−0.05	−0.35
Alfalfa	3.35	2.60	2.70	1.75	+0.60	+0.10	+1.05	−0.15
Double alfalfa	2.60	1.85	1.90	1.60	−0.15	−0.65	+0.25	−0.30
Ammonium sulfate	1.65	1.05	0.75	0.80	−1.10	−1.45	−0.90	−1.10
Sandy soil								
Soil alone	3.45	2.70	3.05	2.75				
Albumin	5.20	2.75	2.65	1.35	+1.75	+0.05	−0.40	−1.40
Casein	5.75	1.80	0.50	1.20	+2.30	−0.90	−2.55	−1.55
Starch	3.40	2.65	2.95	3.30	−0.05	−0.05	−0.10	+0.55
Blood	3.95	0.75	0.40	0.35	+0.50	−1.95	−2.65	−2.40
Dextrose	3.05	2.15	2.00	2.40	−0.40	−0.55	−1.05	−0.35
Alfalfa	3.35	2.50	2.05	2.75	−0.10	−0.20	−1.00	0.00
Ammonium sulfate	2.65	1.60	0.95	1.40	−0.80	−1.10	−2.10	−1.35

Ammonium sulfate, true again to its physiologically acid nature, has been just as consistent in making a drain upon the carbonates as in increasing the soil acidity, which is more evidence of the significance of mineral acids. The two soils behave in a similar manner qualitatively, but there are usually more marked effects produced upon the humus soil.

GENERAL DISCUSSION

The acidities and carbonates have been run in duplicate on duplicate pots and as a rule checks were good. Relative results are shown in each table in

columns to the right marked with a plus or minus sign, according to the effect produced as compared with the soil alone. The soil itself in each case has apparently become less acid after the first sampling, which might signify oxidation of organic acids present in the original soil. It is true, however, that many of the minor variations are considered accidental rather than fundamental. There are errors in mixing the original soil, and again in adding the treatments, and yet again in taking a comparatively small sample from so great a bulk of soil. Results are, therefore, interpreted with these limitations in mind.

Rather than have conclusions drawn from so brief and limited a study some general indications may be pointed out. In the first place the more acid soil has been more active in every respect, indicating that measurable acidity is not a correct index to toxicity. And again no effect which may be attributed to acids has been permanent and consistent except where a mineral acid could be credited for the cause. These permanent effects have resulted from the sulfuric acid liberated from the ammonium sulfate and from the nitric acid accruing from nitrification. Where only organic acids may be presumed to have appeared the effect has been slight or, if temporarily quite appreciable, has soon disappeared. Another effect may be noted in that the basicity resulting from the breaking down of protein is more permanent than any acidity which may result. This is due to the fact that acids are either volatile or are oxidized to carbon dioxide which is volatile. Nitrogenous bases, on the other hand, are continuously active being absorbed and held by the soil until nitrified. In general, then, organic matter seems to act the part of a base rather than of acid, at least to the extent that a given acidity is less detrimental in the presence of an adequate supply of organic materials. Even though organic acids may temporarily take up bases, they are freed again by the complete oxidation of the organic radical.

Theoretically, hydrolysis of 10 tons glucose or starch may produce an acidity equivalent to 5.6 tons of calcium carbonate, if only acetic acid were produced and the charge were complete. It is perfectly evident that nothing like this effect ever occurs. In the same way the change of the one ton of ammonium sulfate, if it were complete would cause an acidity of 1.5 tons, which is only a little more than is actually found in some cases. Likewise the nitrogen of the nitrogenous materials should produce in round numbers about 5.3 tons acidity if the change were complete. But since some ammonia is always present which has neutralizing power and the nitrogen change is never complete, a much smaller effect in increased acidity is produced. The nitrogen of the alfalfa if all changed to nitric acid would produce only about a ton of acidity, while the lime from the same source would be equivalent to nearly half a ton, so that no marked effects would be expected from alfalfa as the data shows. Therefore, from combined data both experimental and theoretical, we may conclude that the addition of organic materials to soils is not likely to have a very decided effect in increasing acidity. In fact, the humified

organic matter may act as a protector of base, by combining with it to form insoluble and non-leachable compounds. Then as oxidation proceeds, the mineral bases thus preserved assist in neutralizing any harmful acidity that may occur. Additional base must be supplied, however, to satisfy the demands of the nitric, sulfuric, or other mineral acids, which may at times tend to accumulate, partly due to the use of physiologically acid fertilizers.

PHYSICAL EFFECTS OF TREATMENTS

There is a physical effect of these different treatments upon the soils which is worthy of note. The albumin and casein pots required less water than the others, due to the failure to evaporate moisture as rapidly. This is in accordance with the nature of colloidal material, such as the various proteins, to bind or hold water. Casein, especially in the presence of alkali, has been found to show marked hydrophylic properties. There is also a tendency for the formation of a grayish incrustation on the pots, and the soil handles poorly, is hard and lumpy, even in the presence of lime. These materials, and also blood-treated soils, filter poorly in the early part of the experiment. Colloidal material passed 2 thicknesses of filter paper. Later blood recovered from this characteristic and gave a clear filtrate. These same materials, especially in the early work, gave a filtrate of a somewhat greenish-yellow color, due doubtless to some organic decomposition product.

Of the two, the humus soil worked rather better, giving a clearer filtrate. The carbohydrate materials on both soils caused marked physical effects, though producing a different condition from that of the proteins. The starch, in particular, caused the soil to remain wet and sticky, of a consistency similar to putty. Starch pots too, although apparently of the same water content, always showed a higher per cent of moisture. The alfalfa-treated pots were in the best physical condition, remaining granular, and in good tilth. The ammonium sulfate had little effect. This factor may be worthy of consideration, therefore, in that the physical properties of a soil depend very much upon the nature of organic materials and fertilizers added, and that such factors in turn greatly influence bacterial activity and the producing power of a soil. The evil effects of some treatments are not overcome even by lime, when added in amounts compatable with practicability.

SUMMARY

1. None of the organic treatments has increased the lime requirement of the soils. The highly nitrogenous materials have rather had the effect of decreasing the acidity. This effect is very marked at the first sampling.

2. Ammonium sulfate, on the other hand, has consistently caused a marked increase in the lime requirement of both soils.

3. The carbohydrate materials have had a small and inconsistent effect upon the soil reaction.

4. The highly nitrogenous organic materials which diminished the acidity tended to protect the carbonates of the soil at the first sampling. Later the same materials used up the limestone quite completely in some cases and to a much greater extent in all cases than did the soil alone.

5. Ammonium sulfate, likewise, very consistently exhausted the lime of the treated soils.

6. The carbohydrates, as is true of the alfalfa also, had no more marked effects in exhausting the carbonates than in increasing the acidity.

7. Ammonification is greater in the absence of lime on both acid soils. A difference in the soil flora is a possible explanation. The ammonification is probably due in part to the activity of molds.

8. Both casein and albumin ammonify more rapidily than does blood which is of higher nitrogen content.

9. Ammonia does not accumulate in the presence of either carbohydrates or alfalfa.

10. Nitrification occurs most rapidly in the presence of lime.

11. Nitrification is slow in starting in the presence of the nitrogenous materials, except blood, probably due to a slight toxicity of accumulated ammonia and exhaustion of nitrates caused by the multiplied flora.

12. No nitrates were found in the presence of the carbohydrates until the end, probably because they were consumed by the organisms of the soil.

13. Taking the sum of nitrates and ammonia there is the greatest action in the presence of lime on the untreated soil, but the reverse is true in most cases for the treated pots.

14. The two soils show as marked differences in their behavior as do the different treatments. The more acid soil is much more active, probably because of greater content of organic matter and a more abundant flora.

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SOLUBLE NON-PROTEIN NITROGEN OF SOIL

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Received for publication December 1, 1918

Sometime ago there was published from this laboratory a description of a method (3) for the determination of the soluble non-protein nitrogen of the soil. Since that time the method has been considerably improved and many determinations have been made with the refined method. It is the object of this paper to report this work. An experiment has been started whose direction the senior author of this paper must discontinue, and, while yet incomplete many results have been obtained which are interesting and valuable and should go on record.

DEVELOPMENT OF THE METHOD

Early in the work it was found that from 96 to 99 per cent of the nitrogen extracted from normal soil by 1 per cent hydrochloric acid, belonged to the class of soluble non-protein nitrogen compounds. Since the small amount of protein nitrogen contained in the acid extract was insignificant when compared to the total soluble non-protein nitrogen, no appreciable error was introduced by its complete inclusion in this group of compounds.

The usual procedure in the old method for the determination of humus was to extract the soil with 1 per cent hydrochloric acid until the wash water from the soil contained no calcium. With that in mind, experiments were planned to determine the proper number of extractions in order to obtain a maximum amount of soluble non-protein nitrogen. Accordingly, two acid and two basic soils were extracted various numbers of times with acid, and total soluble non-protein nitrogen was determined. For the details of this the preceding paper on this subject is referred to. Briefly it consists of extracting the acid-extracted soil with 1.5 to 1.75 per cent sodium hydroxide and the clarification of this extract in a centrifuge running at a speed of 30,000 revolutions per minute. The clear extract is neutralized with sulfuric acid, and then acidified with sufficient tri-chloracetic acid to give finally 2.5 per cent of the latter acid. After filtration total nitrogen is determined in an aliquot of the filtrate, by the micro method (2). The nitrogen, so found, is added to that found in the acid extract.

The results and further remarks will be found in table 1.

Duplicate determinations were made on all the soils but are not included in the table. The duplicates agreed very well, the average derivation from

the mean in all the above experiments being less than 0.0004 gm. nitrogen per 100 gm. soil.

Considering the two acid soils it is observed that acid extraction did not increase the amount of soluble non-protein nitrogen to an appreciable extent. The results for the Carrington silt loam are rather inconsistent. This was

TABLE 1

Soluble non-protein nitrogen from soils extracted various numbers of times with 1 per cent hydrochloric acid

Results expressed as gm. nitrogen per 100 gm. air dry soil

SOIL	NO. OF ACID EXTRACT	TEST FOR Ca	SOLUBLE NON-PROTEIN NITROGEN		
			In acid extract	In alkali extract	Total
			gm.	*gm.*	*gm.*
Calhoun silt loam acid soil with lime requirement 4.5 tons $CaCO_3$	None			0.0333	0.0333
	2	Trace?	0.0045	0.0282	0.0327
	4	Absent	0.0045	0.0280	0.0325
	6	Absent	0.0048	0.0280	0.0328
	8	Absent	0.0048	0.0282	0.0330
Carrington silt loam acid soil with lime requirement 5 tons $CaCO_3$	None			0.0371	0.0371
	2	Trace	0.0034	0.0394	0.0428
	3	Trace	0.0038	0.0375	0.0413
	4	Absent	0.0039	0.0371	0.0410
	5	Absent	0.0040	0.0351	0.0391
	6	Absent	0.0041	0.0357	0.0398
	7	Absent	0.0041	0.0352	0.0393
	8	Absent	0.0041	0.0340	0.0381
Fargo loam basic soil 26.3 tons $CaCO_3$	None			0.0364	0.0364
	2	Very heavy	0.0045	0.0302	0.0347
	4	Heavy	0.0049	0.0347	0.0396
	6	Light	0.0056	0.0404	0.0460
	8	Trace	0.0056	0.0407	0.0463
Marshall silt loam basic soil 4.8 tons $CaCO_3$	None			0.0216	0.0216
	2	Light	0.0025	0.0262	0.0287
	4	Trace	0.0028	0.0278	0.0306
	6	Absent	0.0028	0.0280	0.0308
	8	Absent	0.0028	0.0277	0.0305

the first soil experimented with and the duplicates did not agree as closely as in the remainder of the work.

The results for the soils containing lime show conclusively that in order to obtain all of this class of compounds, acid extraction must be carried to the point where but little, if any calcium can be found in the wash water.

The next point taken up in the refinement of the method was to find out whether nitrates would be determined by this method or whether they would

be entirely eliminated. In a paper published by the senior author about simultaneously with this report it was shown that the ordinary methods for total nitrogen in soils, when used on soil containing 0.6 per cent or more of organic carbon, included the nitrogen of nitrates, while with soils containing less organic matter the nitrates were only partially included. It was suspected, therefore, that the nitrate nitrogen in the acid extract might be only partially included in the total nitrogen determination. An experiment was carried out to test this point. It consisted simply of adding a known amount of nitrate solution to an acid extract of a soil and then digesting it as for total nitrogen. A slight increase in total nitrogen was obtained but not nearly sufficient to account for all the nitrogen added. An effort was then made to reduce all the nitrate nitrogen to ammonia by the addition of iron to the acid solution. In this, success was only attained by the use of excessive amounts of iron, 6 gm. of iron reduced 6 mgm. of nitrate nitrogen completely to ammonia, but 7 gm. failed to reduce 12 mgm. of nitrate nitrogen. Since such large amounts of iron would seriously interfere with the subsequent digestion for total nitrogen, the use of iron was abandoned.

Because of the success Allen (1) has had in the determination of nitrates by the use of Devarda alloy in alkaline solution it was decided to attempt to modify that method for the purpose of this work. After considerable preliminary work the following procedure, which was found to include all nitrate nitrogen, was adopted. The acid extract, with the wash water, which altogether usually occupies a volume of about 600 cc. is made slightly alkaline, to the extent of 2 cc. of saturated sodium hydroxide. Then 2 gm. of devarda alloy is added, the mixture distilled through block tin condensers into standard acid until the residue has a volume of 150 cc. This is transferred to 500 cc. Kjeldahl flasks and then digested in the usual manner for total nitrogen. The nitrogen so obtained plus that from the first distillation gives, of course, the total nitrogen in the acid extract. This method while rather cumbersome has been found the only reliable method for the total nitrogen of acid extracts of soil.

After the method had been perfected as detailed above, it was decided to investigate the non-protein nitrogen on some of the samplings of the soils from the experiment described by Stephenson in the preceding paper. The original plan was to determine this group of compounds in the second and last sampling but it was necessary to discontinue the work after the completion of the analysis of the soils from the second sampling. In the first paper on this method it was shown that carefully purified protein, when added to soil, did not increase the soluble non-protein nitrogen if the soil was immediately subjected to the analysis. Since the commercial preparation of the proteins was used in this pot experiment it was thought best to determine whether the soluble non-protein fraction was increased by these commercial preparations. Also the amount of soluble non-protein nitrogen in the original soil mixtures of all the other materials containing nitrogen, before any decomposition had taken place, was determined.

TABLE 2

Carrington silt loam

All results are expressed in grams per 100 gm. air-dry soil

SOIL NUMBER	TREATMENT	NON-PROTEIN NITROGEN						NH_3 NITROGEN + NO_3 NITROGEN		UNKNOWN NON-PROTEIN NITROGEN	
		In HCl extract		Gm. in alkali extract		Total non-protein nitrogen					
		Original	5 weeks inclusive	Original	5 weeks inclusive	Original	After 5 weeks	Original	After 5 weeks	Original	After 5 weeks
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1 and 2	nothing	0.0037	0.0063	0.0395	0.0349	0.0432	0.0412	0.0027	0.0049	0.0405	0.0363
3 and 4	7 tons lime	0.0037	0.0101	0.0395	0.0300	0.0432	0.0401	0.0027	0.0103	0.0405	0.0298
5 and 6	10 tons egg albumen	0.0087	0.0835	0.0399	0.0454	0.0486	0.1289	0.0027	0.0818	0.0459	0.0471
7 and 8	10 tons egg albumen and 7 tons lime	0.0087	0.0330	0.0399	0.0411	0.0486	0.0741	0.0027	0.0364	0.0459	0.0377
9 and 10	10 tons casein	0.0053	0.1066	0.0413	0.0417	0.0466	0.1483	0.0027	0.0981	0.0439	0.0502
11 and 12	10 tons casein and 7 tons lime	0.0053	0.0527	0.0413	0.0349	0.0466	0.0876	0.0027	0.0569	0.0439	0.0307
13 and 14	10 tons starch	0.0037	0.0026	0.0395	0.0321	0.0432	0.0347	0.0037	0.0014	0.0405	0.0333
15 and 16	10 tons starch and 7 tons lime	0.0037	0.0021	0.0395	0.0306	0.0432	0.0327	0.0027	0.0017	0.0405	0.0310
17 and 18	10 tons dried blood	0.0042	0.0741	0.0389	0.0423	0.0431	0.1164	0.0027	0.0702	0.0404	0.0462
19 and 20	10 tons dried blood and 7 tons lime	0.0042	0.0603	0.0389	0.0376	0.0431	0.0979	0.0027	0.0567	0.0404	0.0412
21 and 22	10 tons dextrose	0.0037	0.0049	0.0395	0.0312	0.0432	0.0361	0.0027	0.0036	0.0405	0.0325
23 and 24	10 tons dextrose and 7 tons lime	0.0037	0.0061	0.0395	0.0285	0.0432	0.0346	0.0027	0.0052	0.0405	0.0294
25 and 26	10 tons alfalfa	0.0073	0.0165	0.0392	0.0331	0.0465	0.0496	0.0027	0.0140	0.0438	0.0356
27 and 28	10 tons alfalfa and 7 tons lime	0.0073	0.0163	0.0392	0.0297	0.0465	0.0460	0.0027	0.0162	0.0438	0.0289
29 and 30	20 tons alfalfa	0.0109	0.0253	0.0395	0.0323	0.0504	0.0576	0.0027	0.0209	0.0477	0.0367
31 and 32	20 tons alfalfa and 7 tons lime	0.0109	0.0215	0.0395	0.0312	0.0504	0.0527	0.0027	0.0172	0.0477	0.0355
33 and 34	1 ton $(NH_4)_2 SO_4$	0.0240	0.0185	0.0389	0.0303	0.0629	0.0488	0.0222	0.0169	0.0397	0.0319
35 and 36	1 ton $(NH_4)_2 SO_4$ and 7 tons lime	0.0240	0.0104	0.0389	0.0276	0.0629	0.0380	0.0222	0.0211	0.0397	0.0169

The results first obtained by the analyses of some of these original mixtures were so much higher than the results obtained from the soils alone that it was immediately suspected that the hydrochloric acid had extracted some protein material which, of course, would be included in the soluble non-protein nitrogen by the regular procedure. The work was, therefore, repeated, and the hydrochloric acid extract as soon as obtained was nearly neutralized with sodium hydroxide and then evaporated down to about 100 cc. under diminished pressure. It was then precipitated by the addition of sufficient trichloracetic acid to give a final strength of 2.5 per cent. The precipitate was filtered and washed with 2.5 per cent trichloracetic acid and the nitrogen determined in the filtrate. This, then, taken as the soluble non-protein nitrogen in the original mixtures, together with that after the 5 weeks incubation in the greenhouse, is given in table 2. Also in this table the *sum* of the nitrate and ammonia nitrogen is given. These results are taken from Stephenson's paper. Since all of the ammonia and nitrate nitrogen is found in the soluble non-protein fraction, the "unknown soluble non-protein" nitrogen has been computed and appears in the last two columns of the table.

In a consideration of the results appearing in table 2 perhaps the most important sets of figures to consider are those in the last two columns, namely what has been called the "unknown soluble non-protein nitrogen." All that we know definitely of this group of compounds is that it is not ammonia or nitrates nor is it protein material. No doubt, part of it belongs to the group of amino acids and also some to the polypeptides. But little is known as to where in the di-, tri-, tetra-peptide, etc. this method draws a line. As a matter of fact there is probably no definite place. The point where precipitation takes place depends no doubt upon the amino acids in the polypeptide, as well as upon the number of amino acids.

In a consideration of the unknown soluble non-protein nitrogen two things are of interest; first, the variation of the different treatments due to the incubation, and second, variation of the results with the different treatments. Perhaps the most noteworthy thing to be seen is that the unknown soluble non-protein nitrogen has decreased during the incubation in every case except in those soils treated with the highly nitrogenous material, albumin, casein and dried blood. Even in the limed soils treated with albumin and casein there is a decrease. In the exceptions noted there is a sufficiently large increase to show there has been a quite different action in these soils. From Stephenson's results in the preceding paper it is seen that in soils 5 and 6 and 9 and 10 the amounts of ammonia are very high while nitrification has not yet started very vigorously. In the corresponding limed pots of the albumin- and casein-treated soils nitrification has proceeded much further and the fact that the soluble non-protein nitrogen in these limed soils is so low means, not that there has been less action, but that there has taken place to a greater extent the assimilation of nitrates and other simple compounds, indicating perhaps that action has been considerably more vigorous here. The

TABLE 3

Carrington sandy loam

All results are expressed in grams per 100 gm. of air-dry soil

SOIL NUMBER	TREATMENT	NON-PROTEIN NITROGEN				TOTAL NON-PROTEIN NITROGEN		NH_3 NITROGEN + NO_3 NITROGEN		NON-PROTEIN NITROGEN NOT KNOWN	
		In HCl extract		In alkali extract							
		Original	After 5 weeks	Original	After 5 weeks	Original	After 5 weeks	Original	After 5 weeks	Original	After 5 weeks
		gm.	gm	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1 and 2	Nothing	0.0028	0.0042	0.0315	0.0316	0.0343	0.0358	0.0011	0.0022	0.0332	0.0336
3 and 4	6 tons lime	0.0028	0.0056	0.0315	0.0272	0.0343	0.0328	0.0011	0.0031	0.0332	0.0297
5 and 6	10 tons egg albumen	0.0042	0.0552	0.0350	0.0350	0.0392	0.0902	0.0011	0.0621	0.0381	0.0281
7 and 8	10 tons egg albumen and 6 tons lime	0.0042	0.0476	0.0350	0.0300	0.0392	0.0776	0.0011	0.0382	0.0381	0.0394
9 and 10	10 tons casein	0.0031	0.0511	0.0344	0.0345	0.0375	0.0856	0.0011	0.0579	0.0364	0.0277
11 and 12	10 tons casein and 6 tons lime	0.0031	0.0418	0.0344	0.0300	0.0375	0.0718	0.0011	0.0254	0.0364	0.0464
13 and 14	10 tons starch	0.0028	0.0038	0.0315	0.0289	0.0343	0.0327	0.0011	0.0011	0.0332	0.0316
15 and 16	10 tons starch and 6 tons lime	0.0028	0.0028	0.0315	0.0270	0.0343	0.0298	0.0011	0.0011	0.0332	0.0287
17 and 18	10 tons dried blood	0.0033	0.0652	0.0323	0.0365	0.0356	0.1017	0.0011	0.0736	0.0345	0.0281
19 and 20	10 tons dried blood and 6 tons lime	0.0033	0.0637	0.0323	0.0317	0.0356	0.0954	0.0011	0.0498	0.0345	0.0456
21 and 22	10 tons dextrose	0.0028	0.0032	0.0315	0.0269	0.0343	0.0301	0.0011	0.0011	0.0332	0.0290
23 and 24	10 tons dextrose and 6 tons lime	0.0028	0.0037	0.0315	0.0266	0.0343	0.0303	0.0011	0.0011	0.0332	0.0292
25 and 26	10 tons alfalfa	0.0056	0.0094	0.0312	0.0274	0.0368	0.0368	0.0011	0.0083	0.0357	0.0287
27 and 28	10 tons alfalfa and 6 tons lime	0.0056	0.0117	0.0312	0.0240	0.0368	0.0357	0.0011	0.0101	0.0357	0.0256
29 and 30	1 ton $(NH_4)_2SO_4$	0.0235	0.0175	0.0312	0.0257	0.0547	0.0432	0.0217	0.0156	0.0330	0.0276
31 and 32	1 ton $(NH_4)_2SO_4$ and 6 tons lime	0.0235	0.0164	0.0312	0.0235	0.0547	0.0399	0.0217	0.0165	0.0330	0.0234

very decided decrease in unknown soluble non-protein in the soils treated with dextrose and starch means the same thing, namely, that the bacteria have used up the simpler nitrogen compounds because of the presence of such large amounts of available energy-producing material.

The results for the more sandy soil appear in table 3.

Upon an inspection of the results in table 3 it is observed that, in general, they are similar to those of the soil higher in organic matter except in an important and striking instance. In the soils treated with the highly nitrogenous materials in the case of the sandy soil, the unlimed soils show a decrease in unknown soluble non-protein nitrogen while the limed show an increase. The opposite was true for the humus soil. Stephenson found that the sandy soil had very poor nitrifying power when compared with the humus soil, and from his tables it is evident that in the sandy soils from this sampling, particularly in the case of the protein treatments, there were very few nitrates, while in the humus soil nitrates in large amounts were present. The explanation for the difference between the two soils in the unknown soluble non-protein nitrogen is probably bound up in the difference in their nitrifying power. Nitrification is, of all the phases of biologic activity in soils, perhaps the most sensitive to soil acidity, or conversely the presence of lime is most necessary for its vigorous function. In the production of nitrates the organism takes into its cell nitrogen, no doubt, from the simpler nitrogenous bodies when these are available. In the case under consideration, in the sandy soil there is not a vigorous nitrifying flora and hence in the limed soil there is no great assimilation of nitrogen. In fact there is not as much assimilation as in the unlimed. It will be observed that in the remainder of the sandy soil pots the unlimed soils show not as much excess unknown soluble non-protein nitrogen over their corresponding limed soils as in the case of the humus soil.

CONCLUSIONS

1. In order to obtain the maximum soluble non-protein nitrogen from basic soils they should be extracted with 1 per cent hydrochloric acid until the wash water from the extracted soils shows no calcium, or at most a trace of it.
2. It is not necessary at all to extract acid soils with 1 per cent acid in the soluble non-protein nitrogen determination.
3. Devarda alloy in weakly alkaline solution has been found a practicable method for the complete reduction of nitrates in the 1 per cent acid extract of soil.
4. The unknown soluble non-protein nitrogen is usually decreased by lime but there are some striking exceptions to this rule.

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CAN WE PREDICT PROBABLE FERTILITY FROM SOIL BIOLOGICAL DATA?

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Received for publication December 20, 1918

Among the many processes active in soils, whereby both mineral and organic materials are rendered available for absorption by the higher plants, none are more important than the normal functioning of bacteria and fungi. In fact we know that any soil, deprived of its microscopic flora, soon becomes a barren waste incapable of producing normal plants. Bacteria and fungi are very low forms of plant life requiring the same mineral elements for their growth and multiplication as do the higher crop plants with which we are more directly interested. Is it not possible, therefore, that there may exist a relationship or a correlation between certain bacterial activities and the actual crop-producing powers of soils? In an endeavor to answer this question, several investigators during the past few years have attempted to correlate the fertility (or crop-producing power) of soils with their abilities to biologically produce ammonia, nitrates or carbon dioxide, and a few soil scientists have been fairly successful in predicting fertility, or lack of it, from such biological data. Brown (3, 4) at the Iowa Agricultural Experiment Station; Lipman (13) at California; Lyon, Bizzell and Conn (18) at New York; Gainey (8) at Kansas; Given (9) at Pennsylvania; Ashby (1) and Russell and Hutchinson (21), in England; and Vogel (22, 23, 24) and several other German investigators, may be cited as having contributed to our knowledge along these lines of work. That there is often little agreement, either in the data obtained or in the conclusions drawn therefrom by these investigators, is seen by a comparison of their several efforts. This is doubtless due, at least in large part, to the dissimilarity of methods employed. As has been inferred, the attempt has been made in the work above cited to in some degree correlate the yields from various soils with their abilities to biologically transform nitrogen compounds. The organic material used has thus usually remained constant in kind and amount while the soils have varied. Conversely, by allowing the soil to remain constant, it is possible to compare the availabilities of different nitrogenous fertilizers (especially organics) biologically, and at present such methods are largely used, either by determining ammonifiability, as proposed by J. G. Lipman (17) or better, by determining nitrifiability as advanced by Lipman and Burgess (14, 15). Purely chemical methods, as the old alkaline-potassium-permanganate method (2), are no longer con-

sidered reliable for this purpose. Of course, the absolute method of determining fertilizer availability is by carefully controlled vegetation tests, but as these are both costly and time-consuming, and as such experiments have shown the validity of the bacteriological tests above indicated, the latter are now very widely used.

As has been pointed out by the writer and by others, Hawaiian soils are decidedly different, physically, chemically, and biologically, from mainland soils. Many more or less unproductive soils are received at this laboratory from time to time with inquiries as to the causes for conditions noted in the field. Unless a preliminary examination shows, at once, the probable cause of infertility, the soil sample is usually run for available potash and phosphoric acid (sometimes for total phosphoric acid), nitrate nitrogen, total nitrogen, the lime requirement (Veitch method) if acid, and the alkali content is determined if an excess of soluble salts is present. Beside these chemical tests, it is always desirable to learn something as regards the biological properties of the soils submitted. The question thus naturally presents itself, "Which of the several biological processes occuring in soils, i.e., ammonification, nitrification, nitrogen fixation, or carbon-dioxide production, is the best to use as a criterion of fertility in our Hawaiian soils?" In an endeavor to answer this question the following work was planned and carried through.

EXPERIMENTAL

About 15 or 20 pounds of surface soil were obtained from each of the following sugar plantations:

Soil no. 2 was probably the most fertile one used, although soils no. 1, 8, 4, 6, and 3 were from very good fields. Soil no. 9 was probably the least fertile, while nos. 5 and 7 were but slightly better. As soon as received at the laboratory, the soils were partially air-dried in the shade and sifted through a 5-mm. mesh sieve, the usual precautions being taken to prevent contamination. The soils were all subjected to the following tests: ammonification, nitrification, nitrogen fixation and total water-soluble nitrogen production. The determinations of the amounts of carbon dioxide formed were not here attempted for it has been recently shown (19) that there is a more or less definite correlation between the amounts of CO_2 produced by a soil's flora from a given amount of organic material and the quantities of ammonia simultaneously evolved, and further, the accurate determination of CO_2 as given off from bacterial cultures is not only most difficult, but also the incubation conditions under which the soils must be held during the test are so artificial as to detract somewhat from the value of data so obtained. We also know that a considerable proportion of the CO_2 evolved from soils is due to mold action (20) and the part played by fungi in soil fertility is at present imperfectly known.

The following fertilizers were used in ascertaining the ammonifying, nitrifying and the total water-soluble nitrogen-producing efficiencies of the

above soils: dried blood (13.5 per cent N), alfalfa meal (3 per cent N), and fish scrap (7.73 per cent N).

Triple portions (50 gm. for the ammonifications and 100 gm. for the nitrifications and total water-soluble nitrogens) of each of the soils were weighed out into sterile glass tumblers and covered with Petri dishes, for each of the three fertilizing materials above given, in so far as the amounts of the soils on hand permitted. As the tables following will show, the samples of soil taken proved to be too small (due to the removal of stones and trash when sifted) always to allow for the use of the three fertilizing materials, hence, in many cases, the fish scrap treatments were omitted.

Three incubation periods were employed for the ammonification, nitrification and total water-soluble nitrogen tests, i.e., 10 days, 20 days and 30 days. At the end of each period one culture of each series was removed and analyzed. The blanks on the untreated soils have in each case been subtracted from the results as tabulated in the tables which follow.

TABLE 1

Soils employed

LABORATORY NUMBER	PLANTATION	*DESCRIPTION OF SOIL
1	Hawaiian Sugar Co.	Red Clay loam; fairly fertile
2	Hawi Mill & P. Co.	Brown silty clay loam; very fertile
3	Laupahoehoe S. Co.	Brown silty clay loam; very good soil
4	Honomu S. Co.	Brown silty clay loam; very good soil.
5	Kipahula S. Co.	Grayish clay loam; poor soil
6	Waipio Substation	Light brown silt loam; fairly fertile
7	Honomu S. Co.	Light brown clay loam; poor; not as good as No. 4
8	H. S. P. A. Exp. Sta., Honolulu	Dark brown clay loam; very good soil
9	Pacific Sugar Mill	Light brown clay loam; poor, acid soil

* The descriptions of these soils are based upon the crops of sugar cane which they are producing in the field with normal fertilization (1000 to 1500 pounds complete fertilizer per acre).

The ammonification results. The usual direct soil culture, or "beaker method," was here employed. To 50 gm. of soil were added 1 gm. of each of the organic materials, irrespective of their nitrogen contents. The incubation temperature was 28°C. As stated above, three different incubation periods were allowed, i.e., 10 days, 20 days and 30 days. At the end of each period one culture using dried blood, one using alfalfa meal and one using fish scrap, were analyzed for ammonia by the usual magnesium oxide distillation method. The results secured at the end of each incubation period and with each fertilizer used are presented in table 2 which follows. In the column "Laboratory Numbers," the numbers refer to the soils employed, and correspond to those given in table 1, while the letters refer to the fertilizing materials used, i.e., A. dried blood; B. alfalfa meal; and C. fish scrap.

The usual incubation period for ammonification studies is from 7 to 10 days and the most common organic material used for this test is finely ground dried blood. For this reason, let us briefly discuss the relative fertilities of the various soils as shown when dried blood was used and the analyses were made at the end of 10 days. Soil no. 2, which is certainly one of the most fertile soils used (if not the very best), heads the list, followed by soils no. 1, 9, 4, 6, 8, 3, 7, and 5. Soil no. 5 is certainly one of the poorest ones and it shows the lowest ability to ammonify, but, on the other hand, soil no. 9 is also very poor while it is third on the list, lacking but a few tenths of a per cent of being next to the best soil noted. Soil no. 3, while it stands near the

TABLE 2

The ammonification results

LABORATORY NUMBER	10-DAY INCUBATION PERIOD		20-DAY INCUBATION PERIOD		30-DAY INCUBATION PERIOD	
	Mgm. added nitrogen ammonified	Per cent added nitrogen ammonified	Mgm. added nitrogen ammonified	Per cent added nitrogen ammonified	Mgm. added nitrogen ammonified	Per cent added nitrogen ammonified
1A	76.4	56.6	50.4	37.3	35.6	26.4
1B	6.7	22.3	3.6	12.0	2.8	9.3
1C	41.4	53.8			24.9	32.3
2A	100.2	74.2	65.5	48.5	59.9	44.4
2B	14.8	49.3	12.0	40.0	5.6	18.7
3A	57.1	42.3	63.0	46.7	46.5	34.4
3B	12.9	43.0			4.8	16.0
4A	74.5	55.2	57.4	42.5	40.0	29.6
4B	9.8	32.7	11.5	38.3	2.5	8.3
5A	26.6	19.7	29.7	22.0	25.8	19.1
5B	1.4	4.7	1.1	3.6	2.2	7.3
5C	16.0	20.8	1.9	2.5	4.4	5.7
6A	68.6	50.8	70.0	51.8	46.5	34.4
6B	11.2	37.3			5.3	17.7
7A	47.6	35.3	61.0	45.2	45.9	34.0
8A	57.7	42.7	15.4	11.4	31.9	23.6
8B	9.5	31.7			1.7	5.6
9A	75.9	56.2	74.2	55.0	80.1	59.3
9B	14.0	46.7	20.7	69.0	21.6	72.0

lower end of the list, is nevertheless a very good producing soil. Many of the intermediate soils are, however, quite close together in the percentages of added nitrogen which they have been able to ammonify.

Where finely-ground alfalfa meal (a vegetable protein material) was used as the source of nitrogen, certain changes in the relative standing of the soils are seen, although no. 2 still heads the list and no. 5 concludes it as before. Soil no. 1, which before was next to no. 2, is now next to the last, while no. 3, which with dried blood did poorly, ranks third. Soil no. 9 holds second place while it was a close third before, notwithstanding the fact that it is a very poor, acid upland soil. It may be said here that there was considerable mold

growth noticed in the no. 9 cultures (especially when dried blood was used), hence probably much of the ammonia here produced was of fungal origin. With alfalfa meal the soils rank as follows: 2, 9, 3, 6, 4, 8, 1, and 5.

Fish scrap was used only in two instances, but here it shows soil no. 1 to be much better than soil no. 5, which is in accordance with fact and with other tests.

As will be seen, in almost all cases the amounts of ammonia found at the end of the 20- and 30-day incubation periods are much lower than those found at the end of 10 days. As the former periods are too long for maximum ammonification, the data presented are probably of little value, although it will be noticed that there is but little change in the relative positions of the different soils throughout the 30 days.

Purified egg albumen, cottonseed meal and tankages, besides the materials herein employed, have in the past been compared by the author with dried blood as to ammonification in Hawaiian soils. The last, however, has been found to give results slightly more in accord with field conditions, and it is now used almost entirely in work of this character.

From the data presented it is evident that ammonification is not, as a rule, suitable in differentiating between good and poor Hawaiian soils, although it may show differences between *very poor* and *very good* soils. Similar conclusions were reached by Kelley (12), also working with Hawaiian soils. He found that, as a rule, good and poor soils alike supported vigorous ammonification, while nitrification was a variable quantity depending more or less upon aeration. This work also bears out the conclusion of Gainey (8), working on Kansas soils, who says, "A study of the data here presented, secured from widely different localities, soil types, and variations in productivity, convinces anyone of the absence of any correlation between yield and ammonia nitrogen content." On the other hand, Brown (3, 4) secures ammonification results in complete agreement with crop yields. He writes, "It would seem, therefore, that there must be some close relationship between the ammonifying power of soils and their crop production."

The nitrification results. Kelley (12) has recently shown that, for nitrification tests, smaller quantities of nitrogen carrying materials are to be preferred to the older methods where the equivalent of from 1 to 2 per cent of dried blood was used. A better differentiation between both fertilizers and soils is possible where amounts of organic materials somewhat in accord with actual field practice are employed. For these reasons 30 mgm. of nitrogen from each of the three organics were employed per culture in these nitrification tests. The actual amounts of each fertilizer required to furnish 30 mgm. of nitrogen were:

	gm.
A. Dried blood (13.5 per cent N)	0.222
B. Alfalfa meal (3.0 per cent N)	1.000
C. Fish scrap (7.73 per cent N)	0.388

The beaker method, employing 100 gm. of soil per culture was used throughout. The incubation temperature was 28°C. At the end of each of the 3 incubation periods (10, 20 and 30 days) one culture from each series was removed and analyzed for nitrates by the modified phenol-disulphonic-acid method (16). All of the results obtained appear below in table 3.

It is usually found that the optimum incubation period for nitrification in soil cultures under laboratory conditions is from 4 to 5 weeks. The three periods, 10, 20 and 30 days, were here chosen in order that the rates of nitrification, as well as the absolute amounts of nitrate formed during one month, might be ascertained. With the exception of the poor soils, no. 5 and 9, it

TABLE 3

The nitrification results

LABORATORY NUMBER	10-DAY INCUBATION PERIOD		20-DAY INCUBATION PERIOD		30-DAY INCUBATION PERIOD	
	Mgm. added nitrogen nitrified	Per cent added nitrogen nitrified	Mgm. added nitrogen nitrified	Per cent added nitrogen nitrified	Mgm. added nitrogen nitrified	Per cent added nitrogen nitrified
1A	4.7	15.7	15.2	50.6	15.2	50.6
1B	3.8	12.7	9.0	30.0	9.0	30.0
1C	4.4	14.7			12.0	40.0
2A	2.5	8.3	13.6	45.3	20.8	69.3
2B	1.8	6.0	8.0	26.7	15.2	50.6
3A	3.2	10.7	12.0	40.0	20.0	66.7
3B	1.8	6.0			12.0	40.0
4A	4.7	15.7	14.0	46.7	18.0	60.0
4B	4.7	15.7	11.0	36.7	12.8	42.7
5A	6.0	20.0	6.0	20.0	4.0	13.3
5B	3.8	12.7	5.6	18.7	7.2	24.0
5C	9.0	30.0	9.4	31.3	5.0	16.7
6A	1.8	6.0	9.0	30.0	17.2	57.3
6B	0.9	3.0			9.6	32.0
7A	4.0	13.3	12.0	40.0	13.6	45.3
8A	4.5	15.0	16.0	53.3	16.8	56.0
8B	6.0	20.0			10.0	33.3
9A	7.0	23.3	4.0	13.3	4.0	13.3
9B	4.4	14.7	5.6	18.7	4.5	15.0

will be noted that there was a steadily increased production of nitrate nitrogen from the tenth to the thirtieth day in almost all cases. Especially is this noticed with the best soils under examination, i.e., no. 2, 3, 4 and 8. With soils 5 and 9 there was a decrease after the first 10-day period in many cases. This was possibly due to denitrification in some cases while in others the rapid evolution of ammonia occurring at first might have had a tendency to correct existing acidity, thus initially rendering conditions more nearly suitable for nitrification.

Let us now arrange the soils in their order of nitrate production (as shown at the end of the 30-day incubation period where dried blood was used).

Beginning with the best, the soils fall in the following sequence: no. 2, 3, 4, 6, 8, 1, and 7, while 5 and 9 happen to be the same, and are the lowest. The first four or five soils thus listed are all of high productivity, and, as table 3 shows, differ but little in the large percentages of added nitrogen nitrified, while no. 5, 9 and 7 are the lowest nitrifiers, and we know them to be the poorest soils.

Where alfalfa meal was used in place of dried blood (but supplying exactly the same amount of nitrogen, 30 mgm.), the soils group themselves in the following order: no. 2, 7, 4, 3, 8, 6, 1, 5, and 9. Soil no. 2 still heads the list, while no. 5 and 9 remain the poorest. The only change of note is in the case of soil no. 7 which here occupies second place where with dried blood it came later. Soil no. 7 is not a productive soil and why here it should occupy a post so near the front cannot be explained unless it was accidentally contaminated, or unless the causes of its infertility are other than low nitrifying efficiency. That the latter is in part true is known to the writer who made a careful study of these two Honomu soils (no. 4 and 7) over a year ago and submitted his findings at that time to the manager of the Honomu Sugar Company. The results of the chemical work performed upon these two soils at that time were as follows:

SOIL	CITRATE-SOLUBLE			TOTAL NITROGEN	LIME REQUIREMENT TONS PER ACRE-FOOT (VEITCH)
	CaO	P_2O_5	K_2O		
	per cent	*per cent*	*per cent*	*per cent*	
1*	0.083	0.0076	0.0118	0.53	7.5
2	0.054	0.0046	0.0087	0.34	12.2

*No. 1 and 2 are those numbered no. 4 and 7 elsewhere in this present paper.

From this work we see that soil no. 7 is in all respects a poorer soil than no. 4. These two samples were taken by our assistant agriculturist, Mr. W. P. Alexander, on July 9, 1917, and were described by him as follows:

Sample 1. Below the ditch is an area which has grown very good cane. It is in a pocket, i.e., there is drainage from 2 slopes. It was the former site of a Hawaiian kuleana. The soil is deep. The sample was taken from 3 separate holes dug 1 foot deep. A bag was laid in the bottom of the hole and a slice of the side of the hole taken with a spade. This soil was collected on the bag. The 3 separate "slices" of soil were mixed, and constitute the sample.

Sample 2. Above the ditch is an area which produced poor cane. It has grown vegetables in the past. The soil is shallow, as it is more like a ridge where this area is located. The sample was secured from 3 separate holes, as above described.

From an examination of the data as given in table 3 it is evident that the nitrification figures, where dried blood is used and an incubation period of 30 days is employed, are a very good comparative measure of the crop-producing powers of the various soils under discussion. It may be stated here that the writer has examined a large number of Island soils for nitrification efficiency, using both dried blood and ammonium sulfate as sources

of nitrogen, and almost invariably the figures secured are in accord with the known productivity of the soils so tested. Dried blood usually gives more comprehensive results than ammonium sulfate, or, in fact, than any of the other materials tried.

The findings of the writer, as here stated, are in accord with results secured by several investigators on the mainland. Gainey (8), working with Kansas soils, writes:

> As pointed out before there is evidently a correlation between nitrification and yield but not between any other two factors under consideration.

Also,

> We believe, therefore, that while there is usually a correlation between nitrifying power and productivity, it does not imply that the processes of nitrification are responsible for yield or that yields on non-fertile soils are limited by the process of nitrification. As to whether high nitrifying powers are the result of high fertility or that both are the result of common factors, there are very few data to indicate. Since it is not impossible that both factors depend upon available plant-food, we would call attention to the work of Lipman (13) who has detected a relation between yields and certain available inorganic elements, also to the work of Fraps (7) who has detected a relation between nitrogen content of soils and their nitrifying powers.

I may state here that there is, in Hawaiian soils, little correlation between nitrogen content and nitrification, doubtless due, in large part, to varying degrees of soil acidity.

Brown (3, 4) of Iowa, Given (9) of Pennsylvania, and Lipman (13) of California, have all been able to show a direct relationship between nitrification and crop production.

Total nitrogen rendered water-soluble. These tests were set up in exactly the same way as were the nitrification cultures—100 gm. of soil in tumblers plus 30 mgm. of the different forms of organic nitrogen. The temperature and periods of incubation were also the same. At the end of each period one culture of each series was removed and, with 400 cc. of nitrogen-free distilled water, washed into a quart Mason jar. The jars were shaken for one hour in a shaking machine then allowed to stand over night when practically all of the soil had settled out leaving the supernatant liquid slightly cloudy. The latter was then carefully and completely siphoned off leaving the moist soil in the bottoms of the jars. The liquids were clarified by passing them through Pasteur-Chamberland clay filters under pressure. The first 75 cc. was discarded. Another 400 cc. of distilled water was now added to the soils in the jars and the same process repeated. Exactly 200 cc. of each first clear filtrate was mixed with exactly 200 cc. of the second extraction of the same soil (which represented 50 gm. of the soil culture). Ten cc. of concentrated sulfuric acid was then added to each solution and the whole reduced to a bulk of about 100 cc. on the water bath. When cool, each solution was transferred to a Kjeldahl flask, about 8 gm. of iron (reduced by hydrogen) was added,

and the whole allowed to stand over night to reduce the nitrates present (Ulsch's (2) modified method). Most of the water was then carefully boiled off, 30 cc. more of strong sulfuric acid was added, together with about 10 gm. of salt mixture (10 parts K_2SO_4, 1 part $FeSO_4$ and $\frac{1}{2}$ part $CuSO_4$) (10) and the whole digested two hours to obtain any soluble organic nitrogen which might have been present. As color was noted in several of the original solutions, it indicated the presence of soluble organic compounds. The digestions were then neutralized and distilled as in the usual Kjeldahl determination for total nitrogen.

TABLE 4

The total nitrogen rendered water-soluble

SOIL NUMBER	10-DAY INCUBATION PERIOD				20-DAY INCUBATION PERIOD				30-DAY INCUBATION PERIOD			
	Mgm. nitrogen from H_2O solutions	Mgm. nitrogen from soils	Total mgm. nitrogen recovered	Per cent nitrogen rendered H_2O soluble	Mgm. nitrogen from H_2O solutions	Mgm. nitrogen from soils	Total mgm. nitrogen recovered	Per cent nitrogen rendered H_2O soluble	Mgm. nitrogen from H_2O solutions	Mgm. nitrogen from soils	Total mgm. nitrogen recovered	Per cent nitrogen rendered H_2O soluble
1A	11.9	8.4	20.3	67.7	14.7	3.6	18.3	61.0	14.0	2.5	16.5	55.0
1B	8.4	2.2	10.6	35.3	11.2	3.4	14.6	48.7	10.5	2.5	13.0	43.3
1C	11.9	6.9	18.8	62.7					12.6	2.2	14.8	49.3
2A	9.1	17.9	27.0	90.0	15.4	14.0	29.4	98.0	19.8	1.4	21.2	70.7
2B	7.7	13.4	21.1	70.3	12.6	9.5	22.1	73.7	15.4	3.3	18.7	62.3
3A	11.9	9.8	21.7	72.3	16.8	7.6	24.4	81.3	16.8	2.8	19.6	65.3
3B	8.4	8.4	16.8	56.0					13.3	4.7	18.0	60.0
4A	12.6	7.5	20.1	67.0	17.5	6.4	23.9	79.7	14.7	2.5	17.2	57.3
4B	9.8	3.9	13.7	45.7	14.0	4.5	18.5	61.7	12.6	3.6	16.2	54.0
5A	7.7	0.5	8.2	27.3	8.4	1.9	10.3	34.3	4.2	1.4	5.6	18.7
5B	6.3	0.5	6.8	22.7	9.1	1.6	10.7	35.7	10.5	2.5	13.0	43.3
5C	9.1	1.2	10.3	34.3	11.9	1.1	13.0	43.3	9.1	1.1	10.2	34.0
6A	9.8	14.5	24.3	81.0	16.1	10.9	27.0	90.0	16.5	1.9	18.4	61.3
6B	7.7	6.4	14.1	47.0					14.0	3.6	17.6	58.7
7A	9.1	6.1	15.2	50.7	14.7	7.6	22.3	74.3	10.5	1.9	12.4	41.3
8A	14.7	15.0	29.7	99.0	16.1	6.7	22.8	76.0	18.2	3.3	21.5	71.7
8B	9.1	5.6	14.7	49.0					13.3	3.6	16.9	56.3
9A	11.2	10.9	22.1	73.7	16.1	15.0	31.1	103.7	18.2	12.0	30.2	100.7
9B	12.6	10.0	22.6	75.3	17.5	12.6	30.1	100.3	14.0	16.0	30.0	100.0

That certain soluble forms of nitrogen (ammonium salts, amido-acids, amines, etc.) are quite strongly adsorbed by soils and are retained at least in part against washing with pure water, is an established fact. Hence the moist soils, from whence the supernatant liquids had been withdrawn, were transferred to copper flasks and distilled with magnesium oxide. The sum of these two portions, minus the blanks on the soils examined, represents probably very closely the total amounts of water-soluble nitrogen formed by the soil flora from the added insoluble organic materials.

The results as secured by this method appear in table 4. The first columns under each incubation period show the mgm. of nitrogen (blanks subtracted)

which were secured in the water extracts; the second columns, the mgm. found in the residual soils after the extractions (blanks subtracted); the third columns, the sum of these two; and in the last columns under each time period appear the percentages of added organic nitrogen rendered soluble.

A perusal of table 4 shows that the highest percentages of organic nitrogen rendered water-soluble were secured at the end of the 20-day incubation period. Under the conditions of this test we measure both the ammonia nitrogen and the nitrate nitrogen produced, as well as the simpler protein compounds and decomposition products which have been rendered water-soluble by the soil bacteria and fungi. That there is a gradual increase in nitrate nitrogen from the first period to the last, and that the reverse is shown for ammonia production, is brought out by comparing the nitrogen in the water-soluble portion (which doubtless contains practically all of the nitrate) with that recovered by distilling the residual soils with magnesia (which, due to adsorption, probably carries a large part of the ammonia). This fact is possibly better shown by comparing the "mgm. of added nitrogen nitrified" in table 3 with the "total mgm. of nitrogen recovered" from the soils as shown in table 4, for both sets of cultures were similar in the essential details, and should be fairly comparable.

It is to be regretted that cultures were not analyzed at the 20-day period for soils 1C, 3B, 6B and 8B. This was due to lack of soil. However, where dried blood was employed as the source of nitrogen (the "A" cultures), a complete series at the 20-day incubation period is before us for discussion. Let us consider the relative percentages of added blood nitrogen rendered soluble by the several soils. Soil no. 9 heads the list with all of the added nitrogen rendered soluble. It will be recalled that this is one of the poorest soils under discussion, also that its ammonification coefficient was similarly high. The table shows us that a large percentage of this soluble nitrogen is here present as ammonia. The soils occur in the following order, depending on their abilities to render blood nitrogen soluble: no. 9, 2, 6, 3, 4, 8, 7, 1, and 5. It is evident that this order does not correspond with the known fertility of these soils although, with the exception of no. 9 and 1, there is a general tendency for the better soils to appear first.

In so far as data are available for the alfalfa meal series (the "B" cultures), the soils appear in exactly the same order as in the blood series, i.e., no. 9, 2, 4, 1, and 5.

From a careful study of table 4 it is evident that the amount of organic nitrogen rendered water-soluble by a soil's flora is hardly a criterion whereby we may judge of its inherent capacity to produce crops, or whereby comparative fertilities may be differentiated, except in a very general way.

Nitrogen fixation. Due to lack of soil, it was impossible to determine the nitrogen-fixing powers of these soils by the beaker, or soil culture, method. Mannite solutions (5) were therefore made up carrying 1 gm. of mannite per 100 cc. Wide-mouthed Erlenmeyer flasks of 500 cc. capacity, each re-

ceiving 100 cc. of solution, were sterilized and, when cool, inoculated with 1 gm. each of the soils under examination. These cultures were incubated 3 weeks at 28°C., after which total nitrogen determinations were made. These figures (minus the amount of nitrogen introduced with each gm. of soil used as an inoculum) together with the appearance of the cultures, appear in table 5.

Let us briefly consider the data as here given. The three poorest soils certainly fixed the least nitrogen, while soils no. 2 and 8, which are certainly among the best soils under discussion, fix the most. Azotobacter forms were entirely absent from cultures 5 and 9, while their presence in 7 was doubtful. All of the other soils, except possibly no. 1, gave cultures showing good Azotobacter surface membranes. These nine soils fall naturally into three classes depending upon their nitrogen-fixing abilities. The first or best is comprised of soils 2 and 8 which fix about 11 mgm. of nitrogen each where 1 gm. of mannite is supplied as the source of energy. To the second class belong

TABLE 5

The nitrogen-fixation results

SOIL NUMBER	MGM. NITROGEN FIXED PER CULTURE (PER 1 GM. MANNITE)	PRESENCE OF AZOTOBACTER
1	5.60	?
2	11.20	+
3	6.44	+
4	7.00	+
5	2.80	−
6	7.28	+
7	3.92	?
8	10.64	+
9	3.60	−

soils 6, 4, 3, and 1. These are able to fix about 6 or 7 mgm. under the same conditions. The third or poorest class composed of soils 5, 7, and 9, are able to secure less than 4 mgm. of atmospheric nitrogen under the conditions imposed.

Arranging the soils in the order of their abilities to fix free nitrogen in mannite solution cultures we have: no. 2, 8, 6, 4, 3, 1, 7, 9, and 5. Where nitrate production (nitrification) was the measure of comparative fertility employed, it will be recalled that the following order was shown: no. 2, 3, 4, 6, 8, 1, 7, 5, and 9, which, considering the slight differences obtaining in both cases between the results secured with soils 6, 4, 3, and 1, is certainly a remarkably close agreement. Brown (3) of Iowa has also shown a very complete agreement between nitrogen fixation and nitrification.

SUMMARY

The object of this work, as stated in the title, was to ascertain whether or not it is possible to predict, with any degree of accuracy, the crop-producing

powers of Hawaiian soils, or rather their relative crop-producing powers, from microbiological data, i.e., ammonification, nitrification, nitrogen fixation, etc., and if so, which is the best criterion to use in routine, comparative tests.

Nine Hawaiian surface soils, all of which had been under sugar cane cultivation for many years, were chosen for this investigation. Two or three of them were of exceptional fertility, three were capable of yielding good average crops, while three produced poor crops of cane even after fair fertilizer applications.

These soils were brought to the laboratory, carefully sifted through a 5-mm. sieve, after being air-dried in the shade, and the following tests made upon them: ammonification, nitrification, total supplied organic nitrogen rendered water-soluble, and nitrogen fixation. Three incubation periods were used, viz., 10, 20, and 30 days, while three different organic forms of nitrogen (dried blood, fine alfalfa meal and fish scrap) were employed. The ordinary precautions against contamination were taken.

The methods used, together with the results obtained, are discussed.

The following conclusions seemed justified:

1. Ammonification tests are not suitable to differentiate between the fertilities of average Hawaiian soils, although they will often show differences between *very poor* and *very good* soils.

2. The abilities of soils to render organic nitrogen (of blood or alfalfa) water-soluble are of no value as measures of their crop-producing powers.

3. Nitrification (soil culture method) is by far the most accurate biological soil test yet perfected for predicting probable fertility. In fact, it is probably the best single test of any description yet developed for ascertaining the comparative crop-producing powers of arable soils. At least, this holds for Hawaiian soils, and it may be added that the writer has tested scores of Island soils by the method for nitrification as given herein, and in only a very few instances have the nitrification coefficients been at variance with the known fertility of the fields from whence the samples were drawn. Of course there are exceptions, and it must not be inferred that nitrification tests are able to take the place of carefully conducted chemical or vegetation experiments. Active nitrification may not be the *cause* of high fertility, yet those conditions which tend to promote rapid nitrification are very evidently identical with those which tend to give us enhanced crop yields. Furthermore, although nitrification tests may be a means of differentiating between good and poor soils, they do not tell us the *causes* of the differences noted, nor do they show us exactly how to *improve conditions* in soils of low productivity. Chemical, physical and plant physiological experiments alone are able to give us this information. A discussion of these phases of soil work falls beyond the limits of this paper.

4. There was a remarkable correlation between the amounts of nitrogen fixed in mannite solution cultures and the known fertilities of the soils studied. There was little difference in the comparative rating of the soils, depending

upon whether nitrification or nitrogen fixation tests were used as the criteria of fertility. Azotobacter species are seldom present in Hawaiian soils of low productivity; the more fertile soils, however, carry several species (6), and in so far as fixation is concerned, compare favorably with mainland soils of similar crop-producing powers.

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TOXICITY OF "ALKALI" SALTS[1]

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Received for publication January 2, 1919

The detrimental effect of the accumulation in soils of the so-called alkali salts on crop growth has long been recognized and the importance of its control appreciated. Considerable work has been done to determine the concentration at which the different salts become toxic to plant growth. The more recent work, notably that of C. B. Lipman, has pointed out the importance of the effect of various alkali salts on bacterial activities. Most of the experiments up to the present time have been carried on with single alkali salts rather than with combinations of salts as they exist under field conditions. Some of the studies have included certain arbitrary combinations. The purpose of the work recorded in the following pages is to attempt to throw more light on this problem under conditions existing in Oregon.

HISTORICAL

As early as 1884 Warington (19) showed that the presence of 0.032 per cent of sodium bi-carbonate retarded nitrification and that in the presence of 0.096 per cent of this salt, nitrification was very slight. Schloesing (18) added various salts to soils in quantities not exceeding 485 part per million, but found no apparent effect on nitrification. Deherain found that sodium chloride became toxic at a concentration of 0.1 per cent and when higher concentrations were used, nitrification ceased. He also found that sodium nitrate may stop nitrification for a time but at higher concentrations stimulates it.

J. G. Lipman (14) showed that sodium chloride was injurious to nitrifying organisms; and that (17) a distinct decrease was produced as the application of sodium chloride was increased. When 0.1 per cent was added, nitrification was greatly diminished. J. G. Lipman and Brown (15) found that nitrifications were accelerated by sodium nitrate. In a later work they found that sodium nitrate increased the accumulation of nitrates in soils but that a

[1] The author wishes to thank Prof. C. V. Ruzek and other members of the department of the Oregon Agricultural College for the helpful suggestions and criticisms in carrying on this work and also Prof. P. E. Brown of Iowa State College in reading the manuscript and making timely alterations.

[2] Submitted to the Faculty of the Oregon Agricultural College in partial fulfillment of the requirements for the degree of Master of Science in the School of Agriculture, June 1918.

certain periodicity in the accumulation existed. This would account for the different results obtained by different investigators. McBeth and Wright (16) found that carbonates, chlorides, and sulfates inhibited nitrification and that the former were more injurious than the latter.

In a recent work Brown and Hitchcock (1) show that nitrification in a normal soil is stimulated by small amounts of sodium chloride, sodium sulfate, magnesium sulfate and by large amounts of calcium carbonate. Their toxic points for a normal soil under laboratory conditions are sodium chloride 0.02 per cent, sodium sulfate 2 per cent. and calcium carbonate between 1½ and 6 per cent. Nitrification in an alkali soil was increased by small amounts of sodium bi-carbonate, sodium carbonate and calcium carbonate. Sodium carbonate and sodium bi-carbonate became toxic at 0.3 per cent and calcium carbonate at 0.6 per cent. When crops were grown on the soil, a similar effect was noted on the crop as in the case of nitrification.

Recent investigation by Kelley (5) on nitrification in semi-arid soils show that 0.03 per cent of sodium carbonate is distinctly toxic to the nitrification of 1 per cent dried blood, but that concentrations as high as 4 per cent had no effect on the nitrification of 1 per cent dried blood. When 0.15 per cent of ammonium sulfate was used, 0.1 per cent of sodium carbonate became toxic to nitrification, but this same amount of sodium carbonate stimulated nitrification when 0.0635 per cent of ammonium sulfate was used. Similar results were obtained when sodium sulfate was used in place of the carbonate.

According to Engberding (2) ammonium sulfate, sodium nitrate, potassium nitrate and caustic lime all increased the bacterial content of soil, but on the other hand decreased the nitrogen fixing power. Lipman, C. B. and Sharp (10) found that 0.5 per cent to 0.6 per cent of sodium chloride was toxic to nitrogen fixation, and that 1.25 per cent of sodium sulfate and 0.4 to 0.5 per cent of sodium carbonate was also toxic. Sodium chloride was the only one that acted as a stimulant at any concentration.

Work by Peck (17) showed that sodium nitrate depressed ammonification while the sulfate, phosphate and carbonate of calcium caused an increase. J. G. Lipman's (15) work showed an increase in ammonification due to sodium nitrate, while work done by C. B. Lipman (8) showed that ammonification was inhibited by the chloride, sulfate and carbonate of sodium. Sodium chloride became toxic at a concentration between 0.1 and 0.2 per cent, Sodium sulfate at 0.4 per cent and sodium carbonate at 2 per cent. A stimuating effect was noticed in some cases for sodium carbonate but none in the case of the sulfate and chloride. In a later work (9) he found the following toxic points: for sodium chloride less than 0.1 per cent, for sodium sulfate 0.35 per cent and for sodium carbonate 0.025 per cent. All except the carbonate acted as stimulants.

Recently C. B. Lipman (11) has demonstrated that there exists as measured by ammonification a true antagonism between sodium chloride and sodium sulfate and between sodium sulfate and sodium carbonate. In a pre-

vious work (7) he showed that the chlorides of calcium, magnesium, potassium and sodium were toxic in the order named. He also found that no antagonism existed between the calcium ion and the magnesium ion or between the calcium and sodium ion. Marked antagonism existed between calcium and potassium, magnesium and sodium and between potassium and sodium.

Greaves (3) states that the salts of sodium chloride, calcium chloride, sodium sulfate and calcium nitrate are very toxic to ammonifying organisms and their presence to any extent in the soil will greatly reduce ammonification. The compounds that are the most active stimulants for higher plants are also the most active for ammonifying organisms.

Harris (4) has carried on quite extensive work to determine the toxicity of alkali salts both alone and in combination to plant growth. In his work he concludes with the following summary:

> Land containing more than the following percentages of soluble salts is probably not suited for ordinary crops without reclamation: in loam soils, chlorides 0.3 per cent, nitrates 0.4 per cent, carbonates 0.5 per cent and sulfates 1 per cent. In coarse sandy soil, chlorides 0.2 per cent, nitrates 0.3 per cent, carbonates 0.3 per cent and sulfates 0.6 per cent

EXPERIMENTAL

The work carried on was divided in three phases, namely:

(a) The determination of the toxic points of sodium chloride, sodium nitrate, sodium carbonate and sodium sulfate on peas and wheat.

(b) The determination of the toxic point for peas and wheat when a combination of alkali salts were used as found by analysis, in field soils.

(c) The determination of the effect of sodium chloride, sodium nitrate, sodium carbonate and sodium sulfate upon ammonification, nitrification and nitrogen fixation.

The soil used was obtained from the Eastern Oregon Branch Experiment Station of Moro, Sherman County, Oregon. This soil is a heavy silt loam soil and is quite extensive in area throughout the Columbia Basin. Under normal conditions it is quite productive and when properly handled produces large crops of small grains. Under dry farming conditions alkali spots are gradually beginning to develop, while under irrigated conditions, due probably to over-irrigation, alkali soon appears.

The alkali salts present in six different soils were determined by chemical analyses. The soils were selected so that one of the sodium salts predominated in each. The analyses are shown in table 2.

In the greenhouse tests, 250 gm. of soil was placed in tumblers and brought up to optimum moisture content. The different percentages of alkali salts were added and after two weeks the seed was planted. The soils were kept at the optimum moisture content throughout the experiment. Five weeks after germination took place the plants were harvested and the dry weight

obtained. The soils used for the greenhouse test were the Eastern Oregon Silt loam and the six soils of different combinations for the series of salt and and salts in combination, respectively.

TABLE 1

Chemical and mechanical composition of the soil

CHEMICAL ANALYSIS		MECHANICAL ANALYSIS	
	per cent		*per cent*
Silica (SiO_2)	53.04	Fine gravel	0.65
Potash (K_2O)	1.18	Coarse sand	1.72
Soda (Na_2O)	2.06	Medium sand	1.50
Lime (CaO)	7.32	Fine sand	10.00
Magnesia (MgO)	3.15	Very fine sand	11.10
Ferric oxide (Fe_2O_3)	10.40	Silt	69.60
Alumina (Al_2O_3)	15.92	Clay	11.27
Phosphorus pentoxide (P_2O_5)	0.28		
Volatile matter	6.56		
Humus	1.95		

TABLE 2

Alkali salts present in soils

	1	2	3	4	5	6
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
KCl	4.00					6.27
Na_2SO_4		4.67	93.40	58.50		
$NaNO_3$		12.96				87.14
Na_2CO_3		75.95		22.9	2.45	4.03
NaCl	81.15	1.46	6.60	18.6		2.56
Na_2PO_4		4.94				
$CaCl_2$	0.25					
$MgCl_2$	7.71					
$CaSO_4$	6.61				4.05	
$NaHCO_3$	0.28				88.26	
$KHCO_3$					1.76	
$CaCO_3$					3.48	

In the bacteriological tests, 100 gm. of soil of the Eastern Oregon Silt loam were used. The soil was placed in tumblers and the different salts were added. The soil then was brought to optimum moisture content and an infusion of fresh soil was added to each to insure vigorous bacterial action. The soil was incubated for four weeks at room temperature, the moisture content being kept constant by the addition of sterile distilled water.

Series 1. The effect of NaCl *on nitrogen fixation, nitrification and ammonification*

Each series was run for nitrogen fixation, nitrification and ammonification and differ only from one another in the percentage of the salt used. All work was done in duplicate and the results are expressed in milligrams per million parts of soil. The arrangement and results of Series 1 are shown in table 3.

TABLE 3

Effect of NaCl *on nitrogen fixation, nitrification and ammonification*

NUMBER	NaCl	NITROGEN FIXATION		NITRIFICATION		AMMONIFICATION	
		Total nitrogen	Average	NO_3	Average	NH_3	Average
	per cent	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m*	*mgm. p. m.*
1	None	341.0		50.8		10.06	
2	None	341.0	341.0	89.2	70.0	9.90	9.9
3	0.001	1,262.0		57.0		7.00	
4	0.001	1,195.0	1228.5	80.9	68.9	6.80	10.2
5	0.003	1,333.0		80.9		10.03	
6	0.003	1,885.0	1609.0	60.4	70.2	10.80	10.5
7	0.005	1,701.0		63.0		7.50	
8	0.005	1,388.0	1544.5	64.0	63.5	6.60	7.0
9	0.010	322.0		69.0		4.80	
10	0.010	101.0	211.5	58.0	63.5	5.99	4.9
11	0.050	68.0		50.0		5.80	
12	0.050	71.0	69.5	37.7	43.8	6.50	6.1
13	0.100	76.0		42.0		5.90	
14	0.100	94.4	85.2	34.8	38.4	6.80	6.3
15	0.200	35.8		44.0		9.00	
16	0.200	7.2	21.5	44.0	44.0	8.40	8.7
17	0.400	14.0		30.1		7.40	
18	0.400	7.2	10.6	35.0	32.5	7.30	7.3

According to these results NaCl in small amounts acted as a stimulant for nitrogen fixation. The greatest amount of nitrogen was fixed at a concentration of 0.003 per cent. At 0.01 per cent a distinct decrease occurred which continued as the concentration was increased. No marked increase in nitrification was shown in any case and at a concentration as low as 0.05 per cent a decrease occurred as compared with the check. Ammonification was not stimulated to any extent by any of the concentrations. A slight increase was shown at 0.003 per cent while all others were lower than the check. The points of toxicity were 0.005 per cent for ammonification and nitrification and 0.01 per cent for nitrogen fixation.

Series 2. The effect of Na_2CO_3 *on nitrogen fixation, nitrification and ammonification*

This series was arranged and carried on similar to Series 1, the only difference being in concentration of the salt used. The concentrations used were higher than in the case of NaCl.

TABLE 4

Effect of Na_2CO_3 *on nitrogen fixation, nitrification and ammonification*

NUMBER	Na_2CO_3	NITROGEN FIXATION		NITRIFICATION		AMMONIFICATION	
		Total nitrogen	Average	NO_2	Average	NH_3	Average
	per cent	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*
1	None	341		50.8		10.05	
2	None	341	341.0	89.2	70.0	9.9	9.90
3	0.01	341		102.1		17.0	
4	0.01	341	341.0	294.0	198.0	16.6	16.00
5	0.03	341		55.0		21.1	
6	0.03	341	341.0	117.4	86.0	7.7	14.40
7	0.05	342		89.0		15.9	
8	0.05	343	342.0	106.2	97.5	7.4	11.60
9	0.10	205		127.0		13.1	
10	0.10	205	205.0	152.0	139.5	13.1	13.10
11	0.20	137		33.4		10.5	
12	0.20	138	137.5	174.0	103.7	10.8	10.60
13	0.50	138		139.0		11.0	
14	0.50	137	137.5	139.0	139.0	13.2	12.10
15	0.80	68		68.8		10.5	
16	0.80	68	68.0	67.0	67.9	10.8	10.60
17	1.00	27		57.6		9.7	
18	1.00	14	20.5	57.0	57.3	10.0	9.80

At the concentrations used in this work Na_2CO_3 showed no stimulating effect on nitrogen fixation. Perhaps, if concentrations as low as those used in the NaCl series, had been used, a stimulating effect might have occurred. This of course would then be at a concentration less than 0.01 per cent. Nitrification was increased by concentration up to 0.5 per cent. Above this it was inhibited when compared with the check. The sodium carbonate increased ammonification at concentration up to as high as 0.8 per cent. The greatest stimulation took place at 0.01 per cent and as the concentration was increased there was a gradual decrease in the ammonification. The toxic points of Na_2CO_3 were 0.02, 0.08 and 0.01 per cent for ammonification, nitrification and nitrogen fixation, respectively.

Series 3. The effect of $NaNO_3$ *on nitrogen fixation, nitrification and ammonification*

This series deals with the effect of sodium nitrate on bacterial activities. The results on the amounts of nitrogen fixed in the case of nitrogen fixation are the amounts above what was added to the soil by the addition of the $NaNO_3$. The same is true for the results given in the case of the nitrates in nitrification.

TABLE 5

Effect of $NaNO_3$ *on nitrogen fixation, nitrification and ammonification*

NUMBER	Na NO_4	NITROGEN FIXATION		NITRIFICATION		AMMONIFICATION	
		Total nitrogen	Average	NO_3	Average	NH_3	Average
	per ceut	*mgm. p. m.*	*mgm p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m*	*mgm. p. m.*
1	None	341.0		50.8		10.06	
2	None	341.0	341.0	69.2	70	9.90	9.9
3	0.01	359.0		69.0		8.60	
4	0.01	72.0	215.5	69.0	69	8.70	88.7
5	0.03	173.2		−23.0		42.10	
6	0.03	158.0	165.6	−13.0	−18	12.60	27.3
7	0.05	129.0		−36.0		37.10	
8	0.05	216.0	172.5	−32.0	−34	37.20	37.2
9	0.10	288.0		−64.0		12.70	
10	0.10	302.0	295.0	−82.0	−73	6.62	9.6
11	0.15	504.0		−219.0		13.00	
12	0.15	647.0	575.5	−223.0		7.45	10.2
13	0.20	1,079.0		−18.0		10.00	
14	0.20	1,150.0	1,114.5	−40.0	−29	10.60	10.3
15	0.30	1,663.0		−420.0		10.10	
16	0.30	1,052.0	1,357.5	−466.0	−443	9.50	9.8
17	0.40	590.0		301.0		7.20	
18	0.40	559.0	559.0	511.0	406	7.20	7.2

Small amounts of $NaNO_3$ had no apparent stimulating effect on nitrogen fixation. Up to a concentration of 0.15 per cent less nitrogen was found than in the checks. As the concentrations increased up to 0.3 per cent the amount of nitrogen fixed increased. At percentages higher than this there was a decided decrease. The results of $NaNO_3$ on nitrification are not very consistent. From these results, it appears that nitrification is not only depressed but that there is actually a loss of nitrate. At the highest concentration, 0.4 per cent there was a gain in nitrates. This result corresponds with those of Dehrain (8) who found that nitrates may stop nitrification for a time, but later stimulate it.

Ammonification in this series was slightly inhibited by a concentration of 0.01 per cent of $NaNO_3$. The greatest increase was with a concentration of

0.05 per cent. At higher concentration there was a gradual falling off of ammonification and at 0.3 per cent it was inhibited. The toxic points of $NaNO_3$ were 0.01 per cent for ammonification and 0.4 per cent for nitrogen fixation.

Series 4. The effect of Na_2SO_4 *on nitrogen fixation, nitrification and ammonification*

In this series the concentration of the salt used is a great deal higher than in the case of any of the other series.

TABLE 6

Effect of Na_2SO_4 *on nitrogen, nitrification and ammonification*

NUMBER	Na_2SO_4	NITROGEN FIXATION		NITRIFICATION		AMMONIFICATION	
		Total nitrogen	Average	NO_3	Average	NH_3	Average
	per cent	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm. p. m.*	*mgm.. p. m.*
1	None	341		50.8		10.06	
2	None	341	341	89.2	70.0	9.90	9.9
3	0.1	1,172		115.0		2.05	
4	0.1	1,058	1115	119.1	117.0	7.10	4.5
5	0.2	1,150		107.0		14.20	
6	0.2	1,224	1187	74.0	90.5	10.20	12.2
7	0.4	3,230		85.9		17.40	
8	0.4	3,826	3528	86.0	86.0	12.20	14.8
9	0.6	1,228		71.0		10.70	
10	0.6	3,971	2599	74.1	72.0	7.40	9.0
11	1.0	2,080		69.0		10.90	
12	1.0	2,504	2292	73.0	71.0	13.10	12.0
13	2.0	2,519		68.8		12.20	
14	2.0	2,373	2446	66.0	67.4	12.10	12.1
15	3.0	3,240		60.0		11.30	
16	3.0	2,988	3114	57.0	58.5	11.90	16.6
17	4.0	1,526		38.0		14.70	
18	4.0	1,385	1455	27.6	32.8	19.70	17.2

These results showed rather conclusively that Na_2SO_4 stimulated nitrogen fixation with all the different percentages used. The maximum occurred with a concentration of 0.4 per cent although the amount found at a concentration of 3 per cent was almost as large. Nitrification was increased by concentrations as high as 0.4 per cent. Above this concentration there was a gradual falling off. None of the concentrations used inhibited ammonification. The maximum amount took place at the highest concentration. Of all the salts used in this work Na_2SO_4 proved to be the least toxic as measure by all three of the bacterial activities studied.

CROP EXPERIMENTS

The crop experiments were carried on in two sets of series, with wheat and with field peas. The one set was carried on to determine the effect of single alkali salts on crop growth, the other when the alkali salts were used in combination as found under field conditions.

Series 5. The effect of single alkali salts on the yield of wheat and of field peas.

In this series wheat and peas were grown on soil that contained varying percentages of NaCl, Na_2CO_3, $NaNO_3$, and $NaSO_4$, respectively. Where no yields are recorded, it was impossible to get the seeds to germinate.

TABLE 7

Crop yields of wheat and of field peas on soil with different percentages of NaCl, Na_2CO_3, $NaNO_3$, *and* Na_2SO_4

NUMBER	SALT	YIELD OF WHEAT				YIELD OF PEAS			
		NaCl	Na_2CO_3	$NaNO_3$	Na_2SO_4	NaCl	Na_2CO_3	$NaNO_3$	Na_2SO_4
	per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	116	116	116	116	221	221	221	221
2	0.1	98	89	118	112	160	172	276	255
3	0.2	80	130	108	71	226	207	275	178
4	0.4	22	106	75	183	000	202	229	229
5	0.6	00	137	49	98	000	000	000	164
6	0.8	00	175	00	116	000	000	000	230
7	1.0	00	95	00	62	000	000	000	236
8	2.0	00	00	00	50	000	000	000	000

According to these results, field peas are more sensitive to all of the alkali salts used than is wheat. NaCl in small amounts stimulated slightly the growth of peas, but at a concentration between 0.2 and 0.4 per cent, it was impossible even to get the seed to germinate. No stimulating effect was found on peas when Na_2CO_3 was used, although crop growth took place at a higher concentration than in the case of the chloride. No growth took place at concentration above 0.4 per cent. As with the case of the chloride, $NaNO_3$ produced an increase in yield when low concentrations were used but at percentages above 0.4 per cent no growth occurred. At the different concentration at which growth did take place there was practically no difference in yield.

The least toxic of the four salts was Na_2SO_4. A stimulating effect was shown in all cases and growth took place at a concentration as high as 1 per cent. The increase in growth in the case of both the nitrate and the sulfate series undoubtedly is due to the nitrogen and sulfur contained in each respectively.

NaCl had no stimulating effect on the growth of wheat, and as the concentration increased the yield gradually decreased. The growth at 0.4 per cent was very poor and above this no germination took place. The falling off of

the yield in the case where Na_2CO_3 was used was not very marked, and fairly good growth took place with a concentration of 1 per cent. At concentration above this the yield decreased very rapidly. No germination took place at 2 per cent. $NaNO_3$ ranked next to the chloride in toxicity. Wheat seemed to be more sensitive to this salt than field peas, and although germinations took place at a concentration as high as 0.6 per cent the yields in most cases were below that of the checks. In the case of Na_2SO_4, the wheat germinated at a concentration of 2 per cent but no stimulating effect was noted as was the case with field peas.

Series 6. The effect on crop yields of alkali salts in combination, NaCl *predominating*

This series and all of the following series contained different percentages of salts as found by analyzing the water-soluble salts of an alkali soil. In this series NaCl was present in the largest amounts. The salts present and the yields of wheat and peas are shown in table 8.

TABLE 8

Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions

NUMBER	TOTAL SALT	NaCl	$CaSO_4$	KCl	$MgCl_2$	$CaCl_2$	$NaHCO_3$	GERMINATION	YIELD OF WHEAT	YIELD OF PEAS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	None	None	None	None	None	None	None	100	84.0	250
2	None	None	None	None	None	None	None	100	115.0	206
3	0.1	0.08	0.006	0.004	0.007	0.0002	0.0003	100	163.6	195
4	0.2	0.16	0.012	0.008	0.014	0.0004	0.0006	75	145.6	202
5	0.4	0.32	0.024	0.016	0.028	0.0008	0.0012	25	53.0	86
6	0.6	0.48	0.036	0.024	0.042	0.0012	0.0018		00.0	00
7	0.8	0.64	0.048	0.032	0.056	0.0016	0.0024		00.0	00
8	1.0	0.80	0.060	0.040	0.070	0.0020	0.0030		00.0	00
9	2.0	1.60	0.120	0.080	0.140	0.0040	0.0060		00.0	00

In this series the field peas made a poor growth at a percentage higher than that where NaCl was used alone. This may be due to the fact that small amount of $CaSO_4$ was present. Wheat on the other hand stopped growth at a lower concentration than in the case where the single salt was used. Both this series and Series 1 and 5 indicate that NaCl is the most toxic of all the salts tested.

Series 7. The effect of crop yields on alkali salts in combination, Na_2CO_3 *predominating*

In this series Na_2CO_3 made up about 75 per cent of the salts and there was about 12 per cent of $NaNO_3$ with small amounts of the chloride and sulfate present.

TABLE 9

Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions

NUMBER	TOTAL SALT	Na_2CO_3	$NaNO_3$	Na_3PO_4	Na_2SO_4	NaCl	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	None	None	None	None	None	None	100	84	250
2	None	None	None	None	None	None	100	115	206
3	0.1	0.075	0.013	0.005	0.00467	0.0014	100	148	175
4	0.2	0.150	0.026	0.010	0.00934	0.0029	100	60	162
5	0.4	0.300	0.052	0.02	0.00868	0.0058	50	75	152
6	0.6	0.450	0.078	0.03	0.02802	0.0087	50	132	140
7	0.8	0.600	0.104	0.04	0.03736	0.0116		122	00
8	1.0	0.750	0.130	0.05	0.04670	0.0146		00	00
9	2.0	1.500	0.260	0.10	0.09340	0.0292		00	00

In this series the peas again showed a greater resistance to the carbonate than when the carbonate was used alone. This perhaps is due to the small quantities of $NaNO_3$. Wheat stopped growth at a concentration lower than when $NaCO_3$ was used alone. Both of these results are in accord with the results in Series 2 and 5. $NaNO_3$ is more favorable to the legume than to wheat.

Series 8. The effect on crop yields of alkali salts in combination, Na_2SO_4 *predominating*

In this series there were two alkali salts present, Na_2SO_4 and NaCl. There was 94 per cent of the sulfate and 6 per cent of the chloride.

TABLE 10

Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions

NUMBER	TOTAL	Na_2SO_4	NaCl	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	per cent	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	None	None	None	100	84.0	250
2	None	None	None	100	115.0	206
3	0.10	0.093	0.006	100	229.6	178
4	0.20	0.186	0.013	100	162.4	238
5	0.40	0.373	0.026	100	150.0	178
6	0.60	0.560	0.039	100	143.0	224
7	0.80	0.747	0.052	100	115.0	117
8	1.00	0.934	0.066	100	90.0	161
9	2.00	1.868	0.132		00.0	00

Crop growth took place in a concentration as high as 0.8 per cent of the total salt without any apparent decrease in yield. At 1 per cent there was a slight decrease in the case of both the wheat and the field peas, and at a

higher concentration no growth took place. The toxic point in this series is lower than where Na_2SO_4 was used alone. This undoubtedly is due to the NaCl present.

Series 9. The effect on crop yields of alkali salts in combination Na_2SO_4 *predominating*

In this series as in Series 8 Na_2SO_4 predominated, but it made up only about 58 per cent of the total salt. The other 42 per cent was made up of the carbonate and chloride of sodium.

TABLE 11

Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions

NUMBER	TOTAL SALT	Na_2SO_4	Na_2CO_3	NaCl	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	None	None	None	None	100	84.0	250
2	None	None	None	None	100	115.0	206
3	0.1	0.058	0.022	0.018	100	132.4	221
4	0.2	0.117	0.045	0.037	50	134.2	90
5	0.4	0.234	0.091	0.074	100	103.5	247
6	0.6	0.351	0.137	0.111	75	23.0	139
7	0.8	0.468	0.183	0.148	25	84.0	48
8	1.0	0.585	0.229	0.186	25	49.0	50
9	2.0	1.170	0.488	0.372		00.0	00

These results show that as in the case of the previous series both peas and wheat germinated at a concentration as high as 1 per cent. The germination and yield, though at the higher concentrations, were considerably less than in series 8. This injurious effect is undoubtedly due to the presence of both the chloride and carbonate.

Series 10. The effect on crop yields of alkali salts in combination $NaHCO_3$ *predominating*

The salts used in this series were chiefly the carbonates and bi-carbonates of sodium, potassium and calcium with a small amount of $CaSO_4$. The bi-carbonate of sodium predominated in this combination.

Peas would not germinate at a concentration of 0.8 per cent and wheat made very little growth at this same concentration. These percentages are lower than the toxic points found when Na_2CO_3 was used alone. These results would indicate that the bi-carbonate is more toxic than the carbonate.

TABLE 12

Yield on wheat and field peas on soil having alkali salts in combination as found under field conditions

NUMBER	TOTAL SALT	$NaHCO_3$	$KHCO_3$	$CaCO_3$	$CaSO_4$	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	None	None	None	None	None	100	84.0	250
2	None	None	None	None	None	100	115.0	206
3	0.1	0.090	0.002	0.003	0.004	50	85.0	96
4	0.2	0.181	0.004	0.006	0.008	75	124.0	144
5	0.4	0.362	0.007	0.012	0.016	100	136.0	206
6	0.6	0.544	0.012	0.018	0.024	100	119.0	196
7	0.8	0.725	0.016	0.024	0.032		81.2	00
8	1.0	0.907	0.021	0.030	0.040		00.0	00
9	2.0	1.814	0.042	0.060	0.080		00.0	00

Series 11. The effect on crop yields of alkali salts in combination $NaNO_3$ *predominating*

In this series the salts used were the nitrate, carbonate and chloride of sodium. Sodium nitrate made up 87 per cent of the total.

TABLE 13

Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions

NUMBER	TOTAL SALT	$NaNO_3$	Na_2CO_3	NaCl	KCl	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	None	None	None	None	None	100	84	250
2	None	None	None	None	None	100	115	206
3	0.1	0.087	0.004	0.002	0.006	100	89	229
4	0.2	0.175	0.008	0.005	0.012	100	110	235
5	0.4	0.348	0.016	0.010	0.024	75	103	133
6	0.6	0.522	0.024	0.015	0.036		00	00
7	0.8	0.696	0.032	0.020	0.048		00	00
8	1.0	0.870	0.040	0.025	0.060		00	00
9	2.0	1.740	0.000	0.051	0.020		00	00

This combination was the most toxic one used and is in accordance with the results found where the individual salts were used. Neither germination nor crop growth took place at a concentration above 0.4 per cent.

DISCUSSION

The results of this investigation on the effect of crop growth and bacterial activities of alkali salts, although carried on under laboratory conditions, can be used to advantage for planning field work on this problem.

The bacteriological tests checked fairly well with the crop growth and in most cases accorded with the results obtained by J. G. Lipman, P. E. Brown, C. B. Lipman and W. P. Kelley, except in the points of toxicity for an individual salt.

The wide variations in the point of toxicity were almost lacking, although the author did not use any of the compounds like dextrose, blood meal or cotton seed meal, $(NH_4)_2SO_4$ for nitrogen fixation, ammonification and nitrification. The use of such material was intentionally avoided so as to compare as closely as practicable, the activities of organisms in the presence of these injurious salts under the actual crop-growing conditions.

The little variation in the degree of toxicity may be due to such causes as: (a) The variation in manipulation in the experimental technique (b) The difference in soil composition (c) and above all the different bacterial flora with varying vigor present in different soils, under varying local climatic conditions.

SUMMARY

1. In order of toxicity the salts rank as follows: NaCl, $NaNO_3$, Na_2CO_3 and $NaSO_4$. The per cent of the anion and not the cation is the determining factor.

2. Small amounts of each of the different salts used stimulated both crop growth and bacterial activities. This amount varied with the crop grown, and the concentrations at which stimulation took place bore the same relationship to each other as did their toxicity points.

3. NaCl became toxic to both ammonification and nitrogen fixation at 0.01 per cent. The toxic point for wheat was 0.4 per cent and for field peas 0.2 per cent.

4. $NaNO_3$ became toxic to ammonification at a concentration of 0.01 per cent and to nitrogen fixation above 0.4 per cent. Small concentrations of $NaNO_3$ proved toxic to nitrification but at a concentration of 0.4 per cent a marked stimulation took place. The toxic points for wheat and for peas were 0.8 and 0.6 per cent, respectively.

5. Na_2CO_3 was toxic to ammonification at a concentration of 0.02 per cent, to nitrification at 0.8 per cent and to nitrogen fixation at 0.01 per cent. For wheat the toxic point was 1 per cent and for peas 0.6 per cent.

6. Na_2SO_4 proved to be the least toxic of all the salts. Neither ammonification or nitrogen fixation was inhibited to any extent at concentrations up to 2 per cent and peas up to between 1 and 2 per cent.

7. The toxicity point as found when salts were used in combination, as under field conditions, checked very closely with the points found when the individual salts were present. The toxic point of the combined salts depended upon the percentages of the chlorides, nitrates, carbonates and sulfates present, and the combination in which they exist. $CaSO_4$, when present, lowered the toxic point of the chloride, carbonate and nitrate of sodium.

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ABNORMAL STEM GROWTH OF SOYBEANS IN SAND CULTURES WITH SHIVE'S THREE-SALT SOLUTION

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Received for publication January 3, 1919

In a recent paper (1) Shive has shown that several monobasic phosphate salts, when added to soil or to solution cultures, are toxic to soybean plants growing in the media. This toxicity not only retarded the growth of the plants but also produced certain specific injuries to the plant tissue. These tests involved three sets of experiments: (a) plants grown in soil cultures to which the salts were added singly in solutions; (b) plants grown in soil cultures to which the phosphates were applied in connection with a complete fertilizer treatment; and (c) plants grown in solution cultures in which the mixed solutions employed in the soil series were used without alteration.

Each of the phosphates used singly in his soil cultures caused specific injury to the soybean plants when the initial concentration of the solution was above one atmosphere, the mono-potassium phosphate being least injurious and the mono-calcium phosphate producing the greatest injury. The plants from a number of soil cultures in which the phosphates were used in connection with a complete fertilizer also suffered pronounced injury, while the plants grown in solution cultures sustained more pronounced injuries than those grown in the corresponding soil cultures.

The nature of the injury sustained by the soybean plants grown by Shive in the different culture media, was identical and appeared to be directly related to some property common to all of the solutions employed. The injury was first marked by a retardation in growth, after which, evidence of specific injury appeared in the form of dark brown discolorations around the margins of the cotyledons. In cases of severe injury the discoloration spread rapidly over the entire surface of the cotyledons and soon appeared on the foliage leaves. Leaf injury appeared first near the margin in the form of small yellowish, round spots which rapidly turned brown. In severe cases the spots gradually increased in size until they covered the entire leaf surface, causing death and the falling of the organ.

In his soil cultures with complete fertilizer rations evidence of disturbed growth did not appear until the third week of the growth period. The group of cultures to which Shive's original three-salt solution alone was added, suffered no specific injury.

The growth period for these cultures extended over a period of 30 days.

During the past two years, the writers have had occasion to study the behavior of the soybean plant growing in sand cultures and supplied with Shive's 3-salt solution having a total initial osmotic concentration of 1.75 atmospheres. Using this 3-salt solution it was desired to determine the proportions of mono-potassium phosphate, calcium nitrate and magnesium sulfate that would give the best growth of the soybean plant at different stages of its development.

Since the dry weight was to be used as a measure of the growth rate during the different periods, it was of course impossible to carry through to maturity the same individuals According to the plan adopted, the first series of cultures was grown for a period of 30 days, when it was harvested and the dry weights determined. A second series of cultures was then started from the same lot of seed as that used for the first series. During the first 30 days these plants of the second series were given a nutrient solution containing the same proportion of the three salts as had been found to give the best growth rate during the first growth period. From the close of the first 30-day period to the end of 60 days the individual cultures of the second series were supplied with the same different proportions of the three salts as were employed in the first series. At the end of 60 days the second series was harvested and from the dry weights the best proportion of salts for the second period was obtained. In a similar manner the plants for the third series were to be grown from the same lot of seed and supplied with the best proportions of salts during the first and second periods, after which they were to be given the differential feeding and harvested at maturity.

Ten days after transplanting the first series, the plants in the cultures receiving solutions high in mono-potassium phosphate began to show signs of specific injury. When viewed from above the first leaves (the cotyledons having been removed) were marked by dark brown spots. When viewed from below these leaves showed a distinct enlargement and reddening of the midribs and veins (plate 1). The leaves that developed later were of a pale green color and exhibited the characteristic reddening of the veins on the under side.

Of this series, culture R_2C_6 gave the largest dry weight yield. This culture was supplied with a solution having two-tenths of its total concentration derived from mono-potassium phosphate, six-tenths from calcium nitrate and two-tenths from magnesium sulfate. After the first series was harvested the second lot of plants was started and all of the cultures were supplied alike with the above mentioned proportions of the three salts used during the first 30-day period, after which the differential feeding was inaugurated. An inspection of the plants at this time revealed no evidence of specific injury although a few plants appeared to have been injured at the point where the cotyledons were removed.

At the middle of the second growth period, when the plants were approximately 45 days old, the specific injuries which had characterized certain

cultures in the first growth period made their appearance. In this instance, however, the injury was not confined to the plants growing in the cultures receiving solutions high in mono-potassium phosphate. As growth proceeded the injury to the leaves became more pronounced and was accompanied by a thickening of the stems to approximately twice the normal size and a gradual cessation of the growth in height. Plate 2 shows the characteristic appearance of the plants at the end of the 60-day growth period. An examination of cross-sections taken through the affected regions, revealed the fact that the increased diameter of the stem was not the result of a thickening of a particular tissue, and showed that growth in thickness had increased in all of the tissue of the swollen stems except the extreme outer layers. The thickening was less pronounced, however, in the pith than in the vascular ring. Starch was abundant in the swollen tissue. The cultures in which the most pronounced injury occurred were as follows:

R_1C_7 with one-tenth of its total concentration derived from mono-potassium phosphate, seven-tenths from calcium nitrate and two-tenths from magnesium sulfate; R_2C_3 with two-tenths from mono-potassium phosphate, three-tenths from calcium nitrate and five-tenths from magnesium sulfate; R_3C_5 with three-tenths from mono-potassium phosphate, five-tenths from calcium nitrate and two-tenths from magnesium sulfate; R_3C_6 with three-tenths from mono-potassium phosphate, six-tenths from calcium nitrate and one-tenth from magnesium sulfate.

Since this injury was present in cultures growing in solutions of such widely varying salt proportions, it would appear that instead of being correlated with a high concentration of a particular salt, the abnormal growth in the soybean plants must have been the result of some property which was common to all of the Shive 3-salt solutions.

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PLATE 1

CHARACTERISTIC ENLARGEMENT OF MIDRIBS AND VEINS OF SOYBEAN PLANTS GROWN IN SAND CULTURES WITH SHIVE'S THREE-SALT SOLUTION. *A* AND *C*, UNDER SIDE; *B*, UPPER SURFACE; *D*, NORMAL LEAF

STEM GROWTH OF SOYBEANS IN SAND CULTURES

A. G. M'CALL, J. B. S. NORTON AND P. E. RICHARDS

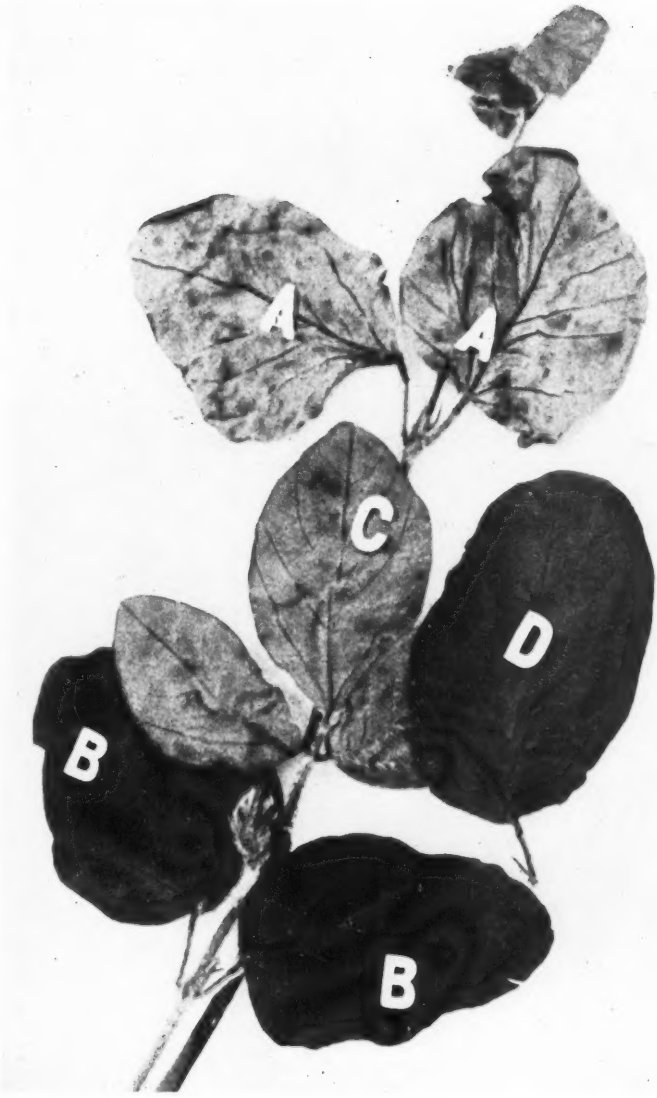

PLATE 2

ABNORMAL STEM DEVELOPMENT OF SOYBEAN PLANTS GROWN IN SAND CULTURES WITH SHIVE'S THREE-SALT SOLUTION

THE DETERMINATION OF TOTAL NITROGEN IN SOILS CONTAINING RATHER LARGE AMOUNTS OF NITRATES

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Received for publication November 25, 1918

In the course of some experimental work on soils kept in the greenhouse, it was found that the nitrate content was rather high, varying from 22 to 153 parts of nitrogen per million. The total nitrogen in the soils varied from 0.125 per cent to 0.156 per cent. The nitrate nitrogen, therefore, amounted to 1.6 per cent of the total nitrogen in the lower case and to 10 per cent in the higher, an amount which should not be overlooked in determining the total nitrogen.

These results confirmed the conclusions of Lyon and Bizzell[2] who found from their own observations and the investigations of others that the nitrate nitrogen even in ordinary cropped and uncropped soils ran all the way from 1 to 50 parts of nitrogen per million, and should certainly be included as a part of the total nitrogen.

There are various methods for determining the total nitrogen in soils, both with and without the inclusion of the nitrates, and several of them were studied to determine their relative accuracy.

The soil taken for analysis contained 145 parts of nitrate nitrogen per million, as determined by the aluminum reduction method, which was the method employed for all the nitrate determinations.

In the first test, four methods for total nitrogen were used, three of which were for total nitrogen including nitrates, and the other simply for total nitrogen. The purpose of the test was to determine whether this later method recovered any or all of the nitrates present. These four methods were the "Ulsch" method, the "Salicylic" method, the "Aluminum" method and the "Hibbard" method. As each of these methods was somewhat modified, a brief statement of procedure will be given here.

Ulsch method. Add 100 cc. ammonia free water to soil, 40 cc. dilute H_2SO_4 (1–2 of H_2O), add 1 gm. iron slowly, boil 5 minutes, add 60 cc. concentrated H_2SO_4, evaporate and drive off fumes; add K_2SO_4 and $CuSO_4$ mixture and digest. Neutralize and determine ammonia.

Salicylic method. 120 cc. concentrated H_2SO_4 containing 8 gm. salicylic-acid. Add slowly 4 gm. zinc dust, drive off fumes, then add K_2SO_4 and $CuSO_4$ mixture and digest.

Hibbard method. Drive off fumes with 75 cc. concentrated H_2SO_4, add K_2SO_4, $CuSO_4$, and $FeSO_4$ mixture and digest.

Aluminum method. Add 150 cc. H_2O, 15 cc. of 10 per cent NaOH and 1 gm. of aluminum to soil. Let digest 24 hours, connecting up Kjéldahl with an Erlenmeyer flask which contains 50 cc. of 10 per cent H_2SO_4 to catch any NH_3 fumes driven off. Pour the 10 per cent H_2SO_4 into the Kjeldahl, add 80 cc. concentrated H_2SO_4, evaporate and drive off fumes, then digest, adding Hibbard mixture.

Twenty-five grams of soil were used in all determinations and each soil was run in quadruplicate.

The widest variation of any one from the average of all the determinations was not over 0.2 of a cc. of N/10 acid.

	AVERAGE NITROGEN	NITROGEN
	gm.	*per cent*
The Ulsch method	0.038353	0.153
The Salicylic method	0.039387	0.158
The Aluminum method	0.039071	0.156
The Hibbard method	0.038784	0.155

This showed at once, that in this particular soil, which contained 10 per cent of its total nitrogen in the form of nitrates, either a reduction of nitrates occurred in the Hibbard method which was not supposed to determine nitrate nitrogen as a part of the total nitrogen, or else none of the methods supposed to include nitrates actually did so.

In the next test a soil which contained no nitrates, as determined by the aluminum reduction method, was employed and total nitrogen determinations were run both with the Hibbard method and the mercury method (the usual method for total nitrogen which is not supposed to include nitrates); with and without the addition of known amounts of nitrates.

(25 GM. SOIL)	AVERAGE NITROGEN	NITRATE NITROGEN RECOVERED
	gm.	*gm.*
Hibbard method soil	0.042906	
Hibbard method soil + 0.00375 gm. nitrogen as KNO_3	0.046584	0.00368
Mercury method soil	0.041400	
Mercury method soil + 0.00375 gm. nitrogen as KNO_3	0.045148	0.00375

These results show conclusively that all the nitrates added to this soil were recovered by the Hibbard and mercury methods. It is evident that in this soil a reduction of nitrates was brought about without the addition of any special reducing agents.

It is possible that some compound in the organic matter of the soil has the same property of reacting with the nitrates as salicylic acid (ortho-hydroxy-

benzoic acid). After such a nitration has been effected, sulphur dioxide, generated by the action of the organic matter on the H_2SO_4, may become the agent reducing the nitrogen compound. In the soil there undoubtedly exist ring compounds such as tyrosine, which have a hydroxy group in the phenyl ring just like salicylic acid, and hence would react similarly. There are also, in all probability, other benzol compounds in the soil which would act in much the same way.

Another test was run to determine whether in soils fairly low in organic matter the nitrates would be recovered by the unmodified methods.

Total nitrogen determinations were made in triplicate on three soils of varying carbon content with and without the addition of 3 mgm. of nitrate nitrogen per sample—an equivalent of 150 parts per million. These determinations were all made by the Hibbard method.

(20 GM. SOIL)	CARBON	NITRATE ADDED	AVERAGE NITROGEN	NITRATE NITROGEN RECOVERED
	per cent	*gm.*	*gm.*	*gm.*
Soil 50181	0.836		0.016252	
Soil 50181	0.836	0.0030	0.019417	0.00316
Soil 50191	0.412		0.009331	
Soil 50191	0.412	0.0030	0.012133	0.00280
Soil 50281	0.188		0.006487	
Soil 50281	0.188	0.0030	0.008126	0.00164

These results show that in soils very low in organic carbon the nitrates are not reduced by this method, and a modified method to include nitrates must be used. However, exept in the case of extremely sandy soils and subsoils, most soils do not run below 0.4 per cent carbon, and on an average they run much higher (0.8 to 3 per cent), so that ordinarily the common methods for total nitrogen will recover the nitrate nitrogen.

The following conclusions may be drawn from this paper:

1. Many soils have a high nitrate content (up to 10 per cent of the total nitrogen) and the recovery of this nitrate is necessary in total nitrogen determinations.

2. If the organic matter of the soil is within the usual average (0.8 to 3.0 per cent), it is not necessary to use the modified methods for total nitrogen, the common unmodified methods (Hibbard or mercury) giving quite as accurate results.

3. Methods for total nitrogen, modified to include the nitrogen of nitrates must be employed, however, if the soils are lower than 0.5 to 0.6 per cent of organic carbon.

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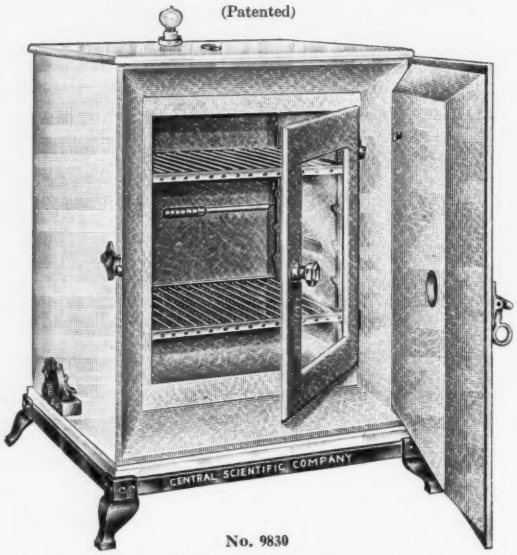

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